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OCTOBER, 1948

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AN UNUSUAL MASKINONGE FROM LITTLE VERMILION LAKE, ONTARIO¹

By G. S. CAMERON²

Abstract

An unusual type of maskinonge found in two lakes in Kenora District, Ontario, is regarded as a hybrid between *Esox masquinongy* and *Esox lucius*. It differs from the typical maskinonge found in the same waters in having a stouter body, longer and deeper head, longer maxillary, and longer fins. It retains dark vertical bars throughout life whereas in the typical form these break up and tend to disappear with age. Of 69 specimens examined, six were of the presumed hybrid type. These all appeared to be sterile. They showed the following *Esox lucius* characters—cheeks totally scaled, head concave interorbitally, cheeks and opercula vividly marked.

The presence of an unusual type of maskinonge in Little Vermilion Lake, Kenora District, Ontario, was brought to general attention in 1945, when it was described as a new species, *Esox amentus*, by Godfrey (3).

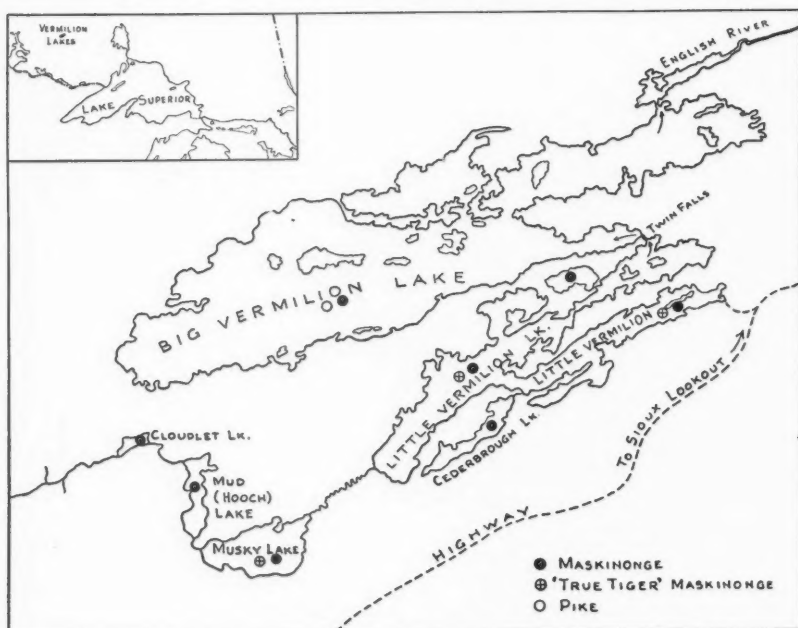
During the summer of 1946, two months were spent on Little Vermilion Lake and a number of other lakes in the vicinity in connection with a taxonomic study of maskinonge undertaken by the Royal Ontario Museum of Zoology with the financial support of the Carling Conservation Club. The accompanying map indicates the location of these lakes, which drain by way of the English River, the Winnipeg River, Lake Winnipeg, and the Nelson River into Hudson Bay.

In the course of these studies, 69 specimens of maskinonge from Little Vermilion Lake, and a smaller connecting lake, known as Maskinonge Lake (Musky Lake), were studied. The study included the making, on each specimen, of 28 measurements of such body proportions as head length, head depth, diameter of eye, length of snout, length of maxillary, body depth and width, caudal peduncle depth and length, and height and base of dorsal, anal, pectoral, and ventral fins. In addition, counts were made of scales in the lateral line, of branchiostegals, and of fin rays. Measurements and counts were made as described by Dymond (1). A description, including a photograph, was made of the markings and color pattern of each specimen. Age was determined also, by scale examination.

¹ Manuscript received March 16, 1948.

Contribution No. 30 of the Royal Ontario Museum of Zoology, Toronto, Ont.

² Junior Zoologist (Seasonal) Royal Ontario Museum of Zoology.



Map of Little Vermilion and surrounding lakes.

Table I presents a comparison of the body proportions and counts of the common and of the so-called 'true tiger' or *amentus* maskinonge. In the case of the common type, only average and extreme ranges are given.

A comparison between a number of these body proportions in the two types is presented graphically in Figs. 1, 2, and 3.

The table and figures indicate several significant differences between the common or typical maskinonge of Little Vermilion and Maskinonge lakes and the so-called 'true tiger' (*amentus*) variant, occurring in the same waters. As compared with the typical form, the variant has a much stouter body (deeper and wider in proportion to length), with a longer and deeper head, much more sharply concave interorbitally, longer maxillary (reaching a vertical through the posterior margin of the eye), and a caudal peduncle both shorter and deeper. The fins are all longer, with larger bases, while the scale count seems slightly lower. Other differences include the complete scaling of the cheek of the variant as compared with the naked lower half of the cheek of the typical form.

The color and markings of the two forms are quite different. Small specimens of the typical form (up to about 30 in. in length) are predominantly bluish green on the sides with distinct dark vertical bars (Fig. 4). Larger fish show a gradual darkening of color, while the markings become gradually

TABLE I

COMPARISON OF BODY PROPORTIONS AND COUNTS OF SCALES AND BRANCHIOSTEGALS OF THE COMMON TYPICAL MASKINONGE OF LITTLE VERMILION AND MASKINONGE LAKES AND OF THE SO-CALLED 'TRUE TIGER' OR *amentus* TYPE FOUND IN THE SAME LAKES

All body proportions listed are expressed as thousandths of standard length; standard length in mm.

	Common type		'True tiger' (<i>amentus</i>) type							
	Average	Range								
Field number	—	—	037	024	075	042	050	035		Mean
Standard length	769	631-1022	850	862	885	904	908	911		887
Head length	276	252-309	315	321	329	303	315	324		318
Head depth	112	092-129	127	115	138	125	143	122		128
Eye	029	024-032	027	028	030	029	026	025		028
Snout	113	104-126	137	137	142	140	142	142		140
Interorbital	067	061-074	072	074	073	075	072	073		073
Maxillary	132	109-145	166	166	167	167	168	170		167
Snout to occiput	190	183-201	222	219	229	226	225	228		225
Body depth	183	163-222	188	209	206	204	193	200		200
Body width	105	089-122	108	115	120	122	120	113		116
Caudal peduncle length	124	103-147	108	122	119	132	123	122		121
depth	074	063-083	075	075	082	076	085	076		078
Dorsal										
rays	22	19- 23	22	22	23	22	23	22		22
height	115	099-129	119	119	134	134	121	128		126
base	120	109-141	132	133	137	128	140	133		134
Anal										
rays	20	18- 22	21	20	20	20	21	21	20/21	
height	114	096-130	119	120	134	127	112	122		122
base	099	090-120	104	104	110	108	098	094		104
Pectoral										
rays	18	16- 19	18	18	18	17	18	18		18
height	115	101-132	114	130	139	133	118	132		128
base	036	030-043	042	042	042	039	035	039		040
Ventral										
rays	12-13	12- 13	12	12	12	12	12	12		12
height	100	089-117	101	112	119	118	110	120		113
base	036	031-040	039	037	037	038	035	038		037
Scales	149	137-156	143	150	143	146	140	145		145
Branchiostegals	17-18	16- 19	19/18	19/20	18/19	18/17	18/17	18/18		17/18

obscured (Fig. 5). The back is often so dark a shade of olive green as to be almost black. This color shades down through bronze to sides that have a ruddy ground color. As a fish ages, the bars break up into obscure blotches, which remain more distinct in the caudal region (Fig. 6). In the largest specimens (over 40 in.) the sides are usually of a uniform dirty brownish color. The belly is usually white, although that of some young maskinonge is marked by faint dark patches. The fins are typically of a brownish color with obscure darker blotches; the fins are often of a vivid red color.

The variants are given the name 'true tiger' because they possess permanent distinct dark crossbars (Fig. 7) traversing light-colored sides, which show a subtle bluish tint. This light color darkens dorsally through a purple hue to a back that is so deep a purple as to appear black. The bars arise from this

black back and slope downwards and forwards, occasionally being broken by distinct dark spots. These markings are sometimes described as 'worm-tracks'. The cheeks and opercula are covered with distinct dark blotches, while the fins are less reddish than those of the typical form, and are faintly spotted.

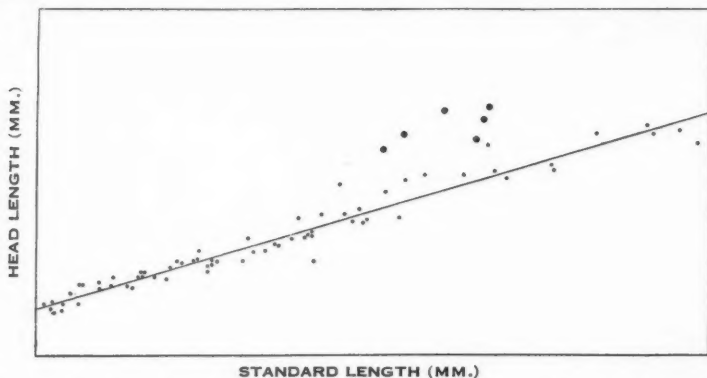


FIG. 1. Diagram showing relation between head length and standard length in typical maskinonge (small dots) and 'tiger' maskinonge (large dots).

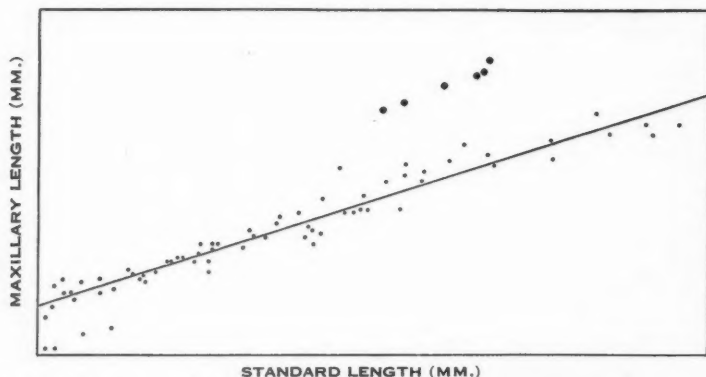


FIG. 2. Diagram showing relation between maxillary length and standard length in typical maskinonge (small dots) and 'tiger' maskinonge (large dots).

Through the co-operation of anglers fishing for maskinonge on Little Vermilion and Maskinonge lakes, and local resort owners, a considerable proportion of the specimens caught and retained were made available for examination. So keen are anglers to exhibit their catch of a rare 'true tiger' that every specimen of this variant taken during the time the study was in progress was photographed and examined. The fact that of the 69 specimens examined only six were of the 'true tiger' type indicates that this type is comparatively rare. This rarity, together with the striking beauty of the

fish makes it a prize eagerly sought after, and may in part explain its reputation for superior fighting qualities. Actually, experienced guides insist that both 'true tiger' and common maskinonge fight with equal vigor.

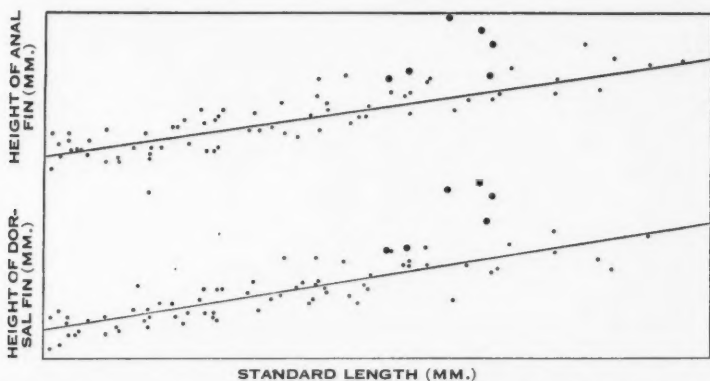


FIG. 3. Diagram showing relation between height of anal and dorsal fins and standard length in typical maskinonge (small dots) and 'tiger' maskinonge (large dots).

A striking feature of these variants was the fact that their gonads were so small and shrivelled as to suggest that they were nonfunctional. The texture was quite different from that of the gonads of normal specimens of the same size.

So far as is known this variant is confined to Little Vermilion and Maskinonge lakes although there were reports of its occurrence in Cliff and Height of Land lakes. Until specimens from these waters can be examined it will not be known whether these are of the same nature or merely vividly marked young of the typical form.

Several of the characters in which the so-called 'true tiger' maskinonge of Little Vermilion and Maskinonge lakes differ from the typical form suggests that it is a hybrid between the common maskinonge (*Esox masquinongy*) and the pike (*Esox lucius*). The pike is not known to occur normally in Little Vermilion and Maskinonge lakes, although it abounds in the lower neighboring lake, Big Vermilion, separated from Little Vermilion by a low falls. Little Vermilion and Maskinonge lakes are joined by a long meandering creek. At high water in spring when these fish spawn it is quite possible that occasional pike may gain entrance to the Maskinonge lakes above.

Some of the considerations that suggest that the 'true tiger' (*amentus*) maskinonge is a *masquinongy-lucius* hybrid are as follows.

It appears to be sterile.

It possesses the following characteristics of *Esox lucius*—cheeks totally scaled, head sharply concave interorbitally, cheeks and opercula vividly marked.

The scale count is intermediate between *lucius* and *masquinongy*.

Presumed *lucius-masquinongy* hybrids are known in other waters and have been produced artificially. Eddy and Surber (3) say that late-maturing pike have been reported as spawning with maskinonge and that evidence of hybridization has been found in the frequent appearance of specimens bearing maskinonge markings but having the cheeks entirely scaled as in the pike.

These authors further report that a large number of maskinonge eggs were successfully fertilized with pike milt at the Nevis Hatchery and that pike eggs were likewise successfully fertilized with maskinonge milt. Some of the resulting fish were reared in the vicinity of the Nevis Hatchery and some in tanks and ponds at the University of Minnesota.

Some of the characters shown by underyearlings of these hybrids have been reported by Eddy (2, pp. 25-27) as follows: "Both of the crosses were heavily barred. Some had the scales absent from the lower part of the cheek, but many showed the lower part of the cheek to be covered partially or entirely by scales." By Sept. 15 the hybrids were between 11 and 12 in. in standard length whereas the pure bred lunge were between 7 and 8 in. in standard length.

The heavy barring and the scaling on the lower part of the cheeks of the artificially produced hybrids correspond to the condition found in the presumed hybrid here reported.

The increased rate of growth and apparent infertility of the presumed hybrid correspond to the condition found by Hubbs and Hubbs (5) in the case of hybrid sunfish.

While the evidence for an increased growth rate in the case of the presumed hybrids reported here is not as great as in the case of the artificial hybrids during their first year there is some indication of it. The six specimens of the *amentus* type, ranging in standard length from 850 to 911 mm. were from 8 to 11 years of age, whereas six typical maskinonge from the same waters 860 to 911 mm. in length were 9 to 14 + years of age.

Four of the seven peculiar maskinonge reported by Seaborn (6, p. 237) were probably pike-maskinonge hybrids as indicated by the barred pattern and the complete scaling of the cheeks.

Acknowledgments

I wish to thank Prof. J. R. Dymond, Director of the Royal Ontario Museum of Zoology, for his guidance in the investigation of this problem, and in the preparation of this report. Gratitude is also due to Mr. Shelley Logier, Royal Ontario Museum of Zoology, who has prepared the figures, and to all those whose co-operation during the investigation was so generously given. These include Mr. Mike Ament, Mr. George More, the late Mr. Howard Noreton, Mr. Archie McDonald, Mr. Ernie Calvert, and numerous others.

PLATE I

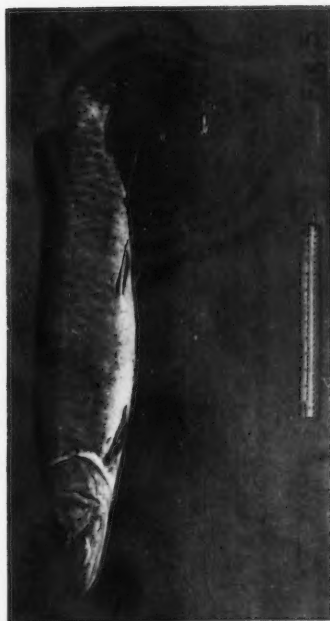
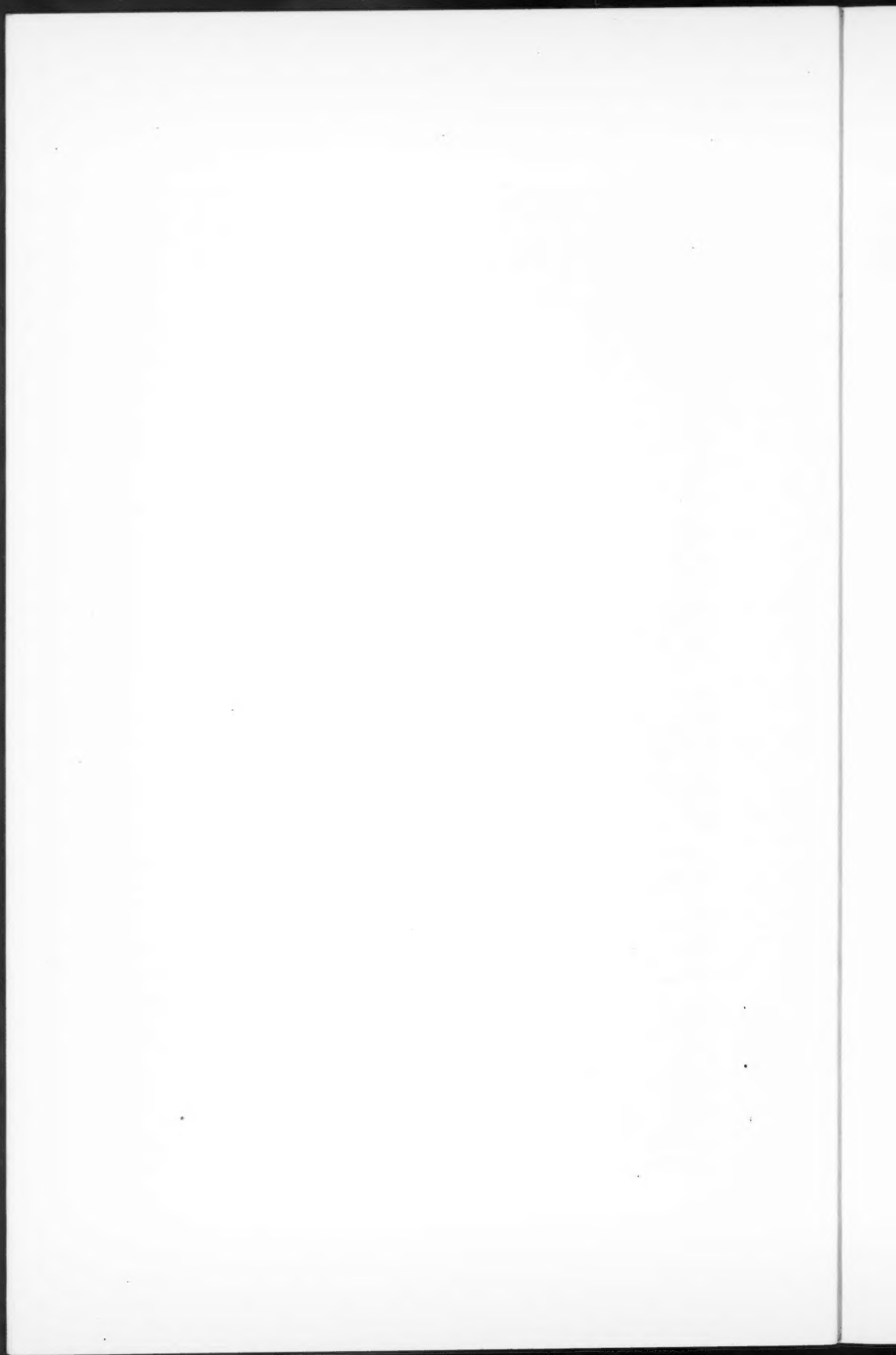


FIG. 4. Typical form, 26 $\frac{1}{2}$ in. long, showing pattern of dark vertical bars. FIG. 5. Typical form, 34 $\frac{11}{16}$ in. long, showing remains of dark vertical bars. FIG. 6. Typical form, 43 $\frac{1}{2}$ in. long, showing only traces of dark vertical bars. FIG. 7. 'True tiger' (amentus) form, 38 $\frac{5}{16}$ in. long, showing persistence of dark vertical bars in larger specimens.



References

1. DYMOND, J. R. The coregonine fishes of northwestern Canada. Trans. Roy. Can. Inst. 24 : 171-231. 1943.
2. EDDY, S. Do muskellunge and pickerel interbreed? Prog. Fish-Cult. No. 48. 1940.
3. EDDY, S. and SURBER, T. Northern fishes. Univ. Minn. Press, Minneapolis. 1943.
4. GODFREY, J., JR. Muskies unlimited. Outdoors. Oct. 1945.
5. HUBBS, C. L. and HUBBS, L. C. The increased growth, predominant maleness and apparent infertility of hybrid sunfishes. Papers Mich. Acad. Sci. 17 : 613-641. 1933.
6. SEABORN, E. Variations in the maskinonge of the Sauble River, Ontario. Copeia, 1937. No. 4. 1937.

THE ANATOMY OF *MONODONTOMERUS DENTIPES* BOH., AN ENTOMOPHAGOUS CHALCID¹

BY GORDON E. BUCHER²

Abstract

The anatomy of the adult of *Monodontomerus dentipes* Boh., a chalcid parasite of sawfly cocoons in Europe and America, is described. An attempt is made to homologize the structures of this highly modified insect with those known in more generalized insects, in the hope of clarifying some questions of chalcid morphology which has been generally neglected by entomologists. The nomenclature employed is of a kind generally acceptable to students of morphology, an effort being made to eliminate the use of terms specific to a limited group of insects. The anatomy of *Monodontomerus* is not widely different from that of phytophagous chalcids described by other authors.

Introduction

This anatomical study of *Monodontomerus dentipes* Boheman was suggested by work at the Dominion Parasite Laboratory, Belleville, Ont., during the summers of 1936-38. This insect was imported, along with many others, in an attempt to combat the spruce sawfly, *Gilpinia hercyniae* Htg., by means of parasites. The chalcidoid group, as a whole, has been neglected morphologically, and it was felt that *Monodontomerus dentipes* would illustrate the anatomy of an entomophagous chalcid.

Considering the enormous numbers of chalcids and their importance as factors in biological control, comparatively little research has been done on their anatomy or morphology. Bugnion (1) published a long paper on *Encyrtus fuscicollis* but chiefly dealt with the postembryonic development and only touched on the adult anatomy. Imms (5, 6), in his papers on the chalcid parasites of Coccidae, investigated the chalcid ovipositor. The most detailed observations have been made by James (7) on *Harmolita graminicola*, a phytophagous chalcid. James apparently ignored the morphological work of Snodgrass, particularly that on the thorax of the Hymenoptera (10), so that some of James's conclusions with regard to the chalcid thorax are in need of revision. The most recent work on the anatomy of the chalcids has been published by Hanna (3, 4).

The genus *Monodontomerus* (Westwood 1833) belongs to the chalcidoid family Callimomidae, whose members are largely parasitic. It contains a number of species of which the best known is *M. aereus* Walker, which was imported into the United States to combat gypsy moth and browntail moth (9).

Monodontomerus dentipes Boh. is widely distributed in Europe as a parasite of several sawflies, particularly *Diprion similis* Htg. In Canada it has not

¹ Manuscript received in original form February 14, 1947 and, as revised, April 20, 1948. Contribution No. 2534, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada. Based on a thesis submitted to the Graduate School, University of Toronto, Toronto, Ont., 1939, in partial fulfilment of the requirements for the degree of Master of Arts.

² Agricultural Scientist, Dominion Parasite Laboratory, Belleville, Ont.

become established on the spruce sawfly but is present locally as a parasite of the introduced pine sawfly *Diprion similis*. It has also been recovered from cocoons of *Lophyrus* sp. on pine in New York State (8).

Material and Technique

Cocoons of *Diprion similis*, containing prepupae of *Monodontomerus dentipes*, were obtained from the Dominion Parasite Laboratory at Belleville and by collections at the Sheridan Nurseries at Oakville, Ont. By storing these in a cool place and incubating them as required, a supply of fresh adults was available at all times.

The usual methods of serial sections and dissections were employed in the study of the anatomy. Dissections were made in a variety of liquids, the most useful being Ringer's solution, 70% alcohol, and a mixture of alcohol and glycerin. A 0.5% solution of acid fuchsin sometimes assisted in distinguishing structures. The skeleton was studied with the aid of specimens cleaned by a 10% solution of sodium hydroxide, bleached by a mixture of potassium chlorate crystals, hydrochloric acid, and alcohol, and stained with acid fuchsin.

A number of techniques were used to obtain serial sections. The most satisfactory sections were obtained by using celloidin as an embedding agent. The best fixation was secured with Lebrun's modification of Carnoy's fixative. Ehrlich's haematoxylin and eosin and iron haematoxylin and erythrosin were the best stains.

General Anatomy

Monodontomerus dentipes is a large insect when compared to most of the other chalcids. Since a number of individuals may mature in one cocoon of the host, the parasite is subject to considerable variation in size, depending on the size of the host and the number of emerging parasites. The following measurements may be considered as only roughly modal. The female is 3.4 mm. long from the front of the head to the tip of the abdomen, but measures 4.1 mm. to the tip of the exerted ovipositor. The female thorax is 0.7 mm. broad and 0.8 mm. deep, while the abdomen is 0.7 mm. broad and slightly deeper. The male is 2.7 mm. long, its thorax 0.6 mm. wide and 0.7 mm. deep, its abdomen 0.5 mm. wide and of about the same depth. Except for variations in size and differences in the genitalia, terminal abdominal segments and related parts, the male is similar to the female. Thus all drawings are of female parts unless otherwise stated.

The cuticle is thick, tough, elastic, and dark green in color with a metallic sheen in certain lights. Its smooth, shiny surface is marred by a fine reticulation forming geometrical figures of inconstant shape and size (Fig. 47). Fine setae are scattered over the surface but occur most frequently at the corners of these figures. Surfaces which are subject to friction have the reticulation suppressed and the setae very fine or absent, as for example the occiput, the mesal surface of the legs, the ventrolateral surfaces of the thorax, and segments

three and four of the abdomen where the hind legs rub. Three sclerites have no reticulation or setae and thus stand out by comparison. These are the mesepimera and the posterior division of the scutellum. Setae are best developed on the dorsolateral portions of the propodeum but are nowhere visible to the naked eye.

The insect is dark green in color except for the red compound eyes, parts of the legs, and antennae. The tibiae are amber; the tarsi are also amber but lighter in color except for the fifth tarsal segments and pretarsi, which are fuscous. Distally the profemora shade from green to amber, but the other femora and all coxae are dark green. The trochanters shade from dark green to medium brown but retain the metallic green luster throughout. The flagella of the antennae are black but in certain lights show a metallic green sheen. The basal segments of the antennae are green.

The Head

THE EXOSKELETON

The head, viewed from the front, is subtriangular in shape, the dorsum being at the base, the mouth parts at the apex, and the compound eyes occupying the sides of the triangle. It is convex in front and concave behind, where the prothorax fits into the occipital region. The head is strictly hypognathous, but upon death, muscular contraction causes it to assume an opisthognathous position, so that the mouth parts are posterior in position and the head itself hides the prosternum (Fig. 24). The head is as broad as any other part of the body, being about 0.9 mm. wide in the female and 0.8 mm. wide in the male.

The Frons

Since the epicranial and frontal sutures are absent in *Monodontomerus*, the areas of the head are not sharply marked out. The frons (Figs. 1, 2, 3, FR) occupies the region between the antennae, stretching from the clypeus to about the median ocellus (MO). It is usually considered to include the median ocellus and the area around it. Since the antennal sockets (ASO) are close together, the frons is narrow. Dorsal to the antennal sockets, the frons is excavated on each side, to accommodate the scapes of the antennae when in repose (FEX).

The Parietals

The regions lateral to the frons are the parietals (Fig. 3, PRTL). They bear the compound eyes, the lateral ocelli (LO), and the antennae. Since the frons is narrow, the parietal regions are large in *Monodontomerus*. The compound eyes (E) are huge organs, occupying practically the whole lateral surfaces of the head. The cuticula is slightly ridged at the border of the compound eyes with the parietal region but a distinct ocular sclerite is not present. Fine short setae are scattered over the surface. The facets of the eye are small and numerous. The lateral ocelli are similar to the median ocellus. All are ellipsoidal but the orientation is different (Fig. 1).

The Clypeus

The anterior tentorial pits (AT) can be seen on each side, a short distance from the middle line near the anteroventral apex of the head (Fig. 3). Joining them is an indistinct groove, which is all that remains of the epistomal suture (ES). Thus the clypeus (CLP) is bounded by the anterior tentorial pits, the suppressed epistomal groove, and the ventral edge of the head capsule. It is quite small in this form. Extending dorsally from the anterior tentorial pits on each side are other ill-defined grooves, which partially mark off a region somewhat more convex than the rest of the anterior surface of the head, and behind which lies the pharynx.

The Genae

On account of the large size of the compound eyes, the genae (Fig. 2, GE) are reduced in size. They lie in a lateral position, between the compound eyes and the ventral edge of the head capsule. A vertical subocular suture (SOS) divides the area into two halves.

The Vertex

The vertex (Figs. 1, 5, VX) is the area on the dorsum of the head. It has one definite posterior boundary, the occipital suture. It is considered as lying behind the ocelli and between the compound eyes. Since the head is wide in *Monodontomerus*, the vertex is correspondingly large.

The Occipital Arch

From a posterior view the occipital arch appears as a concave horseshoelike sclerite, bounded by the occipital and postoccipital sutures (Figs. 5, 6, OCS, POCS). The dorsal part is known as the occiput (OC), while the lateral parts, lying posterior to the genae, are the postgenae (PGE). The occipital and postoccipital sutures are both well developed. The occipital suture separates the occipital arch from the vertex dorsally and from the parietal and genal areas laterally.

The Postocciput and Foramen Magnum

The postoccipital suture separates the occipital arch from the postocciput (POC), the area surrounding the foramen magnum or occipital foramen (FOR).

The occipital foramen is small and, just inside the head, is crossed by the body of the tentorium, which divides it into a dorsal larger aperture, for entrance of the oesophagus, and a ventral smaller neural aperture. At this division, the sides of the foramen are thickened and are inflected posteriorly to articulate with the proepisterna. These are the occipital condyles (OCC).

The Gula

"In the generalized insects . . . there is no ventral sclerotization of the head wall between the foramen magnum and the base of the labium, the submentum being directly continuous with the neck membrane between its lateral attachments to the cranial margins just behind the posterior tentorial pits." (Snodgrass (11)). In *Monodontomerus* the labium is not attached to

the cranium directly but articulates with the maxillae, which in turn articulate with the head near the end of the posterior tentorial arms. The area between this articulation and the foramen magnum is a definite sclerite (Fig. 5, GU), and has been called the gula (7). The morphology of this part is in doubt. Snodgrass considers the gula as a typical coleopterous structure which possibly occurs in some other prognathous insects and which is continuous with the mentum. In this region, certain Hymenoptera have a sclerotized plate called the hypostomal bridge (11), which is formed by mesal extension and union of the postgenae. This has apparently not happened here, since the postoccipital sutures are still in place and divide this area from the postgenae. It seems best to retain the name gula pending further investigation, even though it is not in contact with the submentum. The elongation of the posterior tentorial pits on either side of the gula suggests a growth downward of this region from the lip of the foramen. This would be more like the condition in Coleoptera.

The Subgenae

A suture from the posterior tentorial pits, extending ventrally and laterally on each side to the edge of the cranium above the mandible, cuts off a narrow sclerite around the posterior portion of the gnathal cavity. Its morphology is unknown but it seems to correspond most closely to the subgenal suture of generalized insects (Figs. 5, 6, SGS). The sclerites defined by this suture may be called subgenae (SGE).

THE ENDOSKELETON

The occipital, postoccipital, and subgenal sutures occur internally as inflexions of the cuticle forming minute ridges which doubtless contribute to the support of the exoskeleton.

Along the whole length of the anteromesal border of the compound eyes the cuticula is inflected, forming a deep internal vertical flap or apodeme which separates the ommatidia from the other organs of the head (Fig. 104).

The Tentorium

The tentorium forms the most important endoskeletal support for the head. It consists of a pair of anterior arms and a pair of posterior arms which join together to form the body of the tentorium (Figs. 4, 6). The dorsal arms, which are found in some insects, are absent here.

The anterior tentorial arms (ATA) arise by cuticular invaginations from the anterior tentorial pits (AT) on each side of the clypeus. They extend dorsally and posteriorly forming an arch. Laterally they are expanded into small lamellae (TL), but they lack the median expansion found in some other chalcids (4). The lamellae assist in supporting the supraoesophageal ganglion.

The posterior arms (PTA) arise from the posterior tentorial pits (PT). These are elongate pits, the ventral ends of which lie in the postoccipital sutures but which dorsally invade the postocciput. They show poorly in a fresh specimen but can be found in one cleaned, bleached, and stained. The posterior tentorial arms are much shorter than the anterior arms. They are

directed dorsally and anteriorly to meet the anterior arms. The combined arms on each side continue dorsally and meet finally in a horizontal bar, dividing the foramen magnum into two parts. This bar is the body of the tentorium or corporotentorium (CT). The posterior arms are expanded ventrally towards the articulations of the cardines with the edge of the head capsule.

From the posterior tentorial pits, an internal ridge (ITR) extends to the border of the foramen magnum and supports it. It is revealed externally by a darkening of the chitin. The whole border of the foramen magnum is somewhat thickened for support.

The Head Appendages

THE MOUTH PARTS

The mouth parts consist of the typical parts found in mandibulate insects, and are adapted for biting, rasping, and licking.

The Labrum

The labrum (Figs. 3, 94, LB) is a minute horizontal plate hinging on the clypeus and bearing about five setae. Rarely is it visible from a frontal view; its relations are best seen in sagittal sections (Fig. 106).

The Epipharynx

The epipharynx (Fig. 94, EPH) is a small fold attached to the labrum and forming the roof of the mouth. It is also best seen in sagittal sections (Fig. 106).

The Mandibles

The mandibles are well developed, subtriangular in shape and equal in size (Figs. 2, 3, 6, 13, 14, MD). They are convex anteriorly. From this view they hide most of the other mouth parts (Fig. 3). The opposing margins of each mandible bear two pointed teeth, an apical and a subapical, and, above these, a molar projection. On the posterior surface are two conspicuous skeletal rods which seem to be characteristic features of the chalcidoid mandible; each arises near the apex of a mandibular tooth and ends freely in a club (Figs. 13, 14). Suggestions of these are also seen from the front. The mandibles are articulated with the head in two places. The anterior articulation consists of a condyle on the anterior edge of the gnathal cavity between the subocular suture and the clypeus, which fits into a socket (A) on the anterolateral corner of the mandible. Posteriorly a condyle (C) of the mandible articulates with a depression in the subgena. This articulation is not so well developed as the anterior one. Therefore, while the primary movement of the mandibles is in a transverse plane, a certain flexibility of movement is attained.

The Maxillae

The maxillae (Figs. 2, 5, 7, 8, 9) possess all the parts of the typical maxillary appendages in insects. The basal portion or cardo (CD) of each maxilla

articulates by a shallow socket (A) with a condyle at the lip of the gula (Fig. 5). The stipes (ST) is attached to the distal margin of the cardo. The palpifer of the stipes cannot be distinguished, the four-jointed maxillary palp being borne directly at the distal end of the lateral margin. The galea and lacinia of the maxilla are reduced and somewhat modified from the typical form. The lacinia (LC) articulates with the distal portion of the stipes and assumes a "U" shape, the open part of the "U" being anterior. Thus, components of the lacinia are both lateral and medial. The galea is borne on the lateral side of the lacinia and is attached to the posterior and distal edges of the lacinia. The cuticle, along the line of attachment of the galea to the lacinia, is lighter in color but only slightly more flexible and thus the two parts tend to move and function as a whole. The median portion of the lacinia has a membranous connection with the galea on the medial surface.

The Labium

The labium (Figs. 2, 3, 5, 10, 11, 12) is situated between the maxillae. It consists essentially of a strongly convex plate, the mentum (MT), bearing terminally an unpaired ligula and a pair of three-jointed palps. Proximally the mentum articulates with an ovoid submentum (Fig. 5, SMT), which is only a slight sclerotization of the membrane. Laterally the mentum articulates with the basal part of the lacinia (A). The fleshy ligula (LIG) is borne distally by the mentum. It is a rasping and licking organ. The posterior face is flat but the anterior surface is strongly convex and bears eight or nine serrations which are more rigid than the rest of the organ. The ligula is supported laterally and posteriorly by a pair of sclerites (LSC).

The inner surface of the mentum is occupied by a small lobe, the hypopharynx (Figs. 12, 106, HPH), which is best seen in section as it usually tears away on dissection. Between the hypopharynx and the ligula is the opening of the salivary gland. On either side of the hypopharynx is a skeletal rod (HSC), which lends support to the hypopharynx and labium since the lateral surface of the mentum is only lightly sclerotized.

THE ANTENNAE

The antennae of the male and female are similar except for the difference in size. The scape (Figs. 3, 45, 46, SCP) is the longest segment. It articulates with the head, not directly as is usual in insects, but by a basal segment (BS) as in some other chalcids (6). This appears to be a further modification of the articulating bulb at the base of the scape in many insects. In *Monodontomerus* the bulb has been separated completely and is present as a basal segment.

The antennal sockets (Fig. 3, ASO) are situated close together near the center of the frontal surface of the head. The rim of each socket is strengthened by an internal submarginal ridge appearing on the exterior as an antennal suture (AS). A pivotlike process, the antennifer (AN), on the ventrolateral rim of the antennal socket articulates with the basal segment of the antenna.

The pedicel (Figs. 45, 46, PDC) in *Monodontomerus* is short. Investigation for a possible organ of Johnston was not attempted.

The flagellum, considered as that part of the antenna distal to the pedicel, consists of nine segments. The first or ring segment (RS) is narrow and is found in most chalcids. The remaining segments of the flagellum are subequal with the exception of the terminal one. It is club-shaped, larger than the others, and shows evidence of being compound, since it is divided into three annuli by two sutures.

A mesal extension of the cuticle at the ends of each segment of the funicle reduces the intersegmental channel greatly. From the lip of the anterior foramen thus formed there extends a narrow flange, which articulates with the lip of the posterior foramen of the next segment (Fig. 15, A).

The basal segments of the antenna have scattered setae but the segments of the flagellum beyond the ring segment are covered by numerous setae. They are further characterized by the possession of elongated sensory pits (Fig. 15, SP), which show up light against the black cuticle. These pits are typically arranged in two rows surrounding each segment.

The Prothorax

The prothorax is considerably reduced and the pronotum is only loosely connected to the other parts of the segment, thus leaving the prosternum and propleura to act as a suspensorium for the front legs.

THE PROTERGUM

The protergum (Figs. 16, 17, 18, 20, 22, 23, 24, 25, 26, PRT) is similar in shape to a horse-collar, being produced ventrally to hide the remainder of the prothorax. Posteriorly it covers a considerable portion of the mesothorax and at its dorsolateral corners the cuticle is inflected forming a pair of flanges (PMA) for articulation with the mesonotum. The mesothoracic spiracle opens just below this flange. Anteriorly the edge of the pronotum flares in the middle line and fits against the occiput just above the foramen magnum, thus forming a secondary articulation with the head (PHA). Posteriorly the sides of the protergum are connected by a membranous band which overlies the mesophragma.

THE PROPLEURA

The paired episterna (Figs. 16, 18, 19, 20, 21, 22, 26, EPS) comprise the whole propleura of *Monodontomerus*. They fuse ventrally with the prosternum and curve upwards to a lateral and dorsal position beneath the pronotum. Anteriorly each sclerite bears a heavily sclerotized projection (CV) for articulation with the occipital condyles of the head. These projections probably represent the cervical sclerites which have been fused with the propleura.

THE PROSTERNUM

The prosternum (Figs. 16, 18, 19, 20, 22, 24, 26, STN) is roughly diamond-shaped. At the posterior edge it is reflected upwards between the coxae. James (7) has given the convenient name of reduplication to this inflected part (Figs. 19, 20, 22, PSR).

The arms of the profurca (Figs. 19, 20, 22, AFU) are well developed, arising from the short, low manubrium of the furca (MFU) and the reduplication of the prosternum without an evident pit. They curve outwards to meet the dorsal inflected portions of the episterna, with which they fuse.

The Mesothorax

The mesothorax, being the chief wing-bearing segment, is the largest segment of the thorax. It is highly developed and modified but nevertheless retains a more primitive structure than the other thoracic segments, since most of the sclerites, present in generalized insects, can be identified.

THE MESOTERGUM

The mesotergum is a large compound plate, arched longitudinally and extremely convex, extending far down on each side. It is marked off into surface areas by a number of external sutures, which, with some trouble, can be homologized with those of a typical alinotum (Figs. 23, 25, 27, 28, 29, 33).

The primary suture of the alinotum is a \cap -shaped suture, lying in a posterior position with its apex directed forward, and dividing the notum into an anterior scutum (SCT) and a posterior scutellum (SCL). Internally there is a strong corresponding ridge which, in *Monodontomerus*, is prolonged into a short flap or apodeme (Figs. 29, 33, VR). This primary suture is called the scutoscuteellar suture (VS).

A transverse transscutal suture (TSS) cuts completely through the scutum, setting off two posterolateral areas of the latter from the major scutal area. The parts of the alinotum separated by this suture are frequently called scutum and scutellum and the suture known as the scutoscuteellar suture, particularly by students of Hymenoptera. It is evident that this suture does not correspond to the scutoscuteellar suture, since the latter is also present. It may also be pointed out, that, typically, the scutellum is a plate cut off completely from the wing articulations, while the transscutal suture divides the scutum into an area associated with the anterior notal wing process and one associated with the posterior notal wing process. Since the posterolateral areas of the scutum, defined by the transscutal suture, seem to be without an acceptable name, they may be called postscutum (PSCT), for convenience. They correspond to the prescutellum of Hanna (4).

The anterior area of the scutum is divided into three parts by two sutures extending forwards and outwards from the transscutal suture. These are known as lateral sutures or parapsidal furrows (PF) and separate the dorso-lateral plates or parapsides (PAR) from the median scutum. These sutures

are apparently identical with the convergent sutures or notaulices of other forms. Under the mistaken idea that the parapsidal furrows were discontinuous parts of the transverse prescutal suture turned posteriorly, some morphologists named the area between them prescutum. This is erroneous since the parapsidal furrows and prescutal suture are both present in some Tenthridinidae. There is no transverse prescutal suture or corresponding prescutal plate in *Monodontomerus*.

The parts of the alinotum may be summarized as follows. A scutoscuteellar suture separates the scutellum from the scutum. The scutum is divided by a transscutal suture into an anterior portion and a posterolateral portion, the postscutum. The anterior portion is further subdivided into a single median scutum and lateral parapsides by a pair of parapsidal furrows.

The phragmanotum or postnotum (Figs. 27, 33, PN) is the other major division of the typical pterothorax. In *Monodontomerus* it is completely hidden. The parts of the alinotum may now be considered in more detail.

The Scutum

The scutum (Figs. 23, 25, 27, 28, 29) is a large median plate bearing in front the prephragma (Figs. 27, 29, 31, 1PH). The line of division probably represents the antecostal suture (Fig. 27, ACS) but the acrotergite before the antecostal suture cannot be recognized.

The parapsidal furrows, separating the scutum from the parapsides, are well developed. Internally they form wellmarked ridges (Fig. 29, PR) which are produced into flanges particularly where they meet the prephragma. At this point the scutum bulges somewhat, forming the chief surface (MPA) for articulation with the pronotum.

The Parapsides

From their dorsal connection with the scutum the parapsides (Figs. 23, 25, 27, 28, 29, PAR) curve outwards and downwards to meet the pleura, and thus form a prealar bridge (Figs. 23, 27). Along this lower margin the cuticle is inflected forming a flange (Fig. 29, PFL). The parapsides are cut off from the flange by a submarginal external ridge (PSR). Each parapsis bears a lateral backwardly directed plate which may be called the anterior alar plate (AAP). At the line of fusion of this plate with the parapsis, the latter is prolonged backwards into a short rim (PER). The anterior alar plate, which begins in a vertical plane, flares laterally at the posteroventral corner, forming the anterior notal wing process (ANP) for articulation with the first axillary sclerite.

The tegulae (Figs. 23, 24, 25, 27, TEG) are convex sclerites embracing the bases of the front wings and loosely articulated along the lower edge of the anterior alar plate.

The Postscutum

The postscutum is divided into two dorsolateral areas by the forwardly projecting scutoscuteellar suture. Each postscutal plate (Figs. 23, 25, 27, 28, 29, PSCT) is partially divided into two regions by a short suture (PSS),

extending part way into the plate from the scutoscuteellar suture. Internally a small ridge corresponds to this suture. The contour of the plate is complicated. It roughly takes the form of an "S" sloping downwards and backwards from the transscutal groove and sloping downwards, outwards, and forwards from the scutoscuteellar suture, the suture of the postscutum lying in the excavation so formed. Each postscutal plate bears a posterior alar plate (PAP) corresponding to the anterior alar plate of the parapsis. At the junction of the two, the postscutal plate bears a heavy flange (SFL) which, however, does not reach the notal margin. The posteroventral angle of the alar plate flares laterally, forming a large projecting spine, the posterior notal wing process (PNP), at the base of which the third axillary sclerite articulates.

As mentioned above, the postscutum is separated from the scutum and parapsides by a transscutal suture. In most insects this suture is defined internally by a strong ridge lending support to the mesonotum. In *Monodontomerus* and certain other chalcids (7) this suture forms a zone of weakness and movement, the two parts of the scutum being joined by membranous or semimembranous integument. This probably led James erroneously to name this the scutoscuteellar suture. Internally there is no strong transverse ridge, but the postscutal plate on each side bears a weak apodeme (Figs. 28, 29, SPS) directed forward to lie in a horizontal position beneath the corresponding parapsis. Since their resemblance to true phragmata is strong, these may be termed pseudophragmata.

The Scutellum

The scutellum (Figs. 23, 25, 27, 28, 29, 33, SCL) is a large plate somewhat more convex than the remainder of the mesonotum, occupying a posterior median position, and being defined from the scutum by the scutoscuteellar suture which internally forms a well-marked ridge. The posterior third of the scutellum is set off from the rest by a transscutellar suture (TSCS) which internally forms a very fine low ridge. This posterior plate is characterized by the absence of reticulation and setae and thus is very shiny. The posterior part of the scutellum is traversed by a wide submarginal groove in which the cuticle is thrown into folds, forming a row of wide pits separated by narrow elevations. The marginal part of the plate is a production of the scutellum overhanging the metanotum.

The Postnotum

The postnotum (Figs. 27, 33, PN) of *Monodontomerus* is a narrow flexible plate, weakly attached to the scutellum in front and the metanotum behind, and entirely hidden from view by these structures. Anteriorly it bears an apodeme, lying horizontally beneath the scutellum, which may be termed a pseudophragma (PPS). Posteriorly the postnotum bears the large postphragma (2PH), the posterior attachment of the dorsal longitudinal muscles. The phragma is highly convex with deep lateral areas. It extends backwards and downwards to underlie the metanotum and propodeum and almost reaches the articulation of the petiole with the latter. On each side the postnotum

articulates with the flexible postalar bridge, which extends downwards to connect with the mesepimeron. The postalar bridge (Figs. 23, 27, 28, AB) consists of a dorsal portion of definite shape and considerable rigidity and a ventral portion rather variably marked and comparatively flexible, the line of division indicating a zone of movement.

THE MESOPECTUS

The mesosternum and mesopleura are combined into a compound structure, to which Snodgrass has given the name pectus. The mesopectus in *Monodontomerus* is a large, deep, boat-shaped structure, divided by a transverse suture into an anterior area or prepectus (PRP) and a posterior area, which is further subdivided by a median ventral longitudinal groove into two identical halves. Each half is divided by an oblique pleural suture (PS) into an upper epimeron (EPM) and a ventrolateral sternoepisternal plate.

The Prepectus

The prepectus (Figs. 23, 24, 26; 27, 30, 31, 32, PRP) is a narrow unpaired sclerite set off from the mesopectus by a flexible suture. Dorsolaterally it expands and its dorsal margin on each side articulates with the parapsis and forms the prealar bridge. Internally it bears anterior, posterior, and dorsal submarginal low ridges (Figs. 19, 30, IRP). There are no corresponding external sutures but the line of each ridge is indicated by the greater density of the skeleton. These lines, along with an oblique folding of the cuticle, mark off a visible external triangle on the lateral surface (Fig. 23, TP).

The Mesosternum

The mesosternum (Figs. 24, 26, 30, 31, STN'') is a poorly defined area in *Monodontomerus*. A median longitudinal sternal groove divides it into two halves. This sternal groove marks the inflection of the sternum to form the furcal base. Since the base of the furca is large, almost the whole of the sternum may have been so inflected and the remainder may be represented here merely by the suture. This, however, is pure conjecture and until further evidence has been presented, it is best to consider the sternum as that area occupying a ventral position in the mesopectus. Posteriorly the sternum is reflected dorsally between the bases of the coxae to form a posterior reduplication (MSR) as mentioned above for the prothorax. At the base of the reduplication a definite pit appears, marking the posterior inflection of the sternal apophyses (FP). A pair of spines (Figs. 28, 30, MSRA), borne by the reduplication of the sternum, form a secondary articulation for the mesocoxae.

The Episternum

The mesosternum is not separated from the episternum, which, therefore, must be considered as the lateral part of the combined plate. The episternum (Figs. 23, 27, 32, EPS) is separated by an oblique suture (PS) from the epimeron. This suture stretches from the coxal articulation (A) towards the pleural wing process (PWP), but, before reaching the latter, it bends forwards,

completely separating the episternum from any part in the formation of the wing process. It is beyond the scope of this paper to determine whether this is, in whole or in part, homologous with the true pleural sutures of more generalized insects.

The Epimeron

The epimeron (Figs. 23, 24, 25, 26, 27, 30, 31, 32, EPM), at its anterodorsal angle, is prolonged into a low extended pleural wing process (PWP). The remaining dorsal edge is inflected and produced into a spine for articulation with the postalar bridge (Figs. 27, 32, EP). The contour of the epimeron is complex in the dorsal region; the reader is referred to Fig. 23, where shading has been used to indicate depression. The epimeron is characteristic in having no reticulation or setae. Its shiny surface, however, is marred by a dimple (D) about the middle of the plate.

THE ENDOSKELETON

Internally the mesopectus is variously strengthened. Its anterior margin is inflected forming an apodeme. The dorsal margin is similarly inflected, forming the spine (EP) for articulation with the postalar bridge. The horizontal part of the pleural suture bears an internal ridge (Figs. 27, 28, 30, 32, IPR) which joins with the spine. The oblique portion of the pleural suture is without an internal ridge. The pleural wing process is also strengthened by a ridge (IWR), which in turn unites with this complex.

The Mesofurca

The endosternum of the mesothorax arises by the inflection of the sternum along the middle line, forming a longitudinal vertical plate sometimes called the manubrium or shaft (Figs. 19, 30, 32, MFU). The manubrium forks dorsally, giving off a lateral arm (AFU) at each side, so that the whole structure, the furca, roughly takes the shape of a "Y" and supports the mesothoracic ganglion. Anteriorly the furcal arms are joined by an arch (FA), which overlies the ganglion. Each arm proceeds forward and upwards towards the epimeral spine, to which it is attached by a tendon. In generalized insects the arms of the furca articulate with the pleural process, a projection of the pleural ridge. This suggests that in *Monodontomerus* the epimeral spine represents some part of the pleural ridge. Anatomical evidence reveals it as merely an infolding of the edge of the epimeron. Since, as has been mentioned above, the pleural ridge joins the spine, there are, doubtless, some pleural elements incorporated in it and thus it is not surprising that the furcal arm unites with it.

The Metathorax and Propodeum

The metathorax is greatly reduced in *Monodontomerus*, consisting of a bandlike metanotum and a metapectus which includes both sternal and episternal elements. As in other clistogastrous Hymenoptera, the first abdominal segment has been incorporated with part of the metathorax to form the propodeum which will, therefore, be considered along with the metathorax.

THE METANOTUM

The metanotum (Figs. 23, 25, 38, 39, 40, 41, MN) is a narrow transverse sclerite, lying between the scutellum and the propodeum, and being loosely articulated to the latter and to the concealed postnotum in front. At the sides, it dips abruptly forming a vertical alar plate which bears the pointed anterior notal wing process (ANP) and the rounded posterior notal wing process (PNP). A heavy ridge separates the metanotum from its alar projection. The cuticula of the metanotum is thrown into external folds of fairly constant character marking off wide shallow trenches. The metanotum bears no phragmata.

The metanotum articulates with the postalar bridge on each side by means of a small sclerite (Figs. 27, 28, 68, X), which meets the metanotum in the region of the anterior notal wing process.

THE METAPECTUS

The metapectus (Figs. 23, 26, 34, 39, 40, 41) consists of the combined sternal and pleural regions of the metathorax.

The Episternum

The episternum (EPS) may be considered as the triangular portion of the metapectus visible from the side. Dorsally it is separated from the propodeum by a longitudinal pleural suture (PS), which stretches from the coxal articulation to the pleural wing process (PWP). Internally this suture forms the pleural ridge (IPR) with which the metafurcal arms join. Thus, it seems to be the true pleural suture; and, therefore, the plate below must be episternum, while the epimeron is fused above with the propodeum.

The Metasternum

The metasternum (STN''') consists of a narrow sclerite in front of the hind coxae. It is continuous at the sides with the episterna. Posteriorly it curves upwards between the hind coxae as the reduplication of the metasternum (MTR), and meets the propodeum just below the lower lip of the propodeal foramen.

THE PROPODEUM

The propodeum (Figs. 23, 25, 38, 39, 40, 41, PD) is a large dorsal sclerite, consisting of the first abdominal segment fused with part of the metathorax. The author believes that both the metapostnotum and metepimera are included in the propodeum of *Monodontomerus*. At the sides it is produced forward to form the pleural wing process (PWP). Posteriorly there is a large foramen (Figs. 40, 41, FOP) in the propodeum into which the petiole fits. It is thought that, being an abdominal segment, the propodeum is composed of a dorsal and a ventral plate, the latter being represented by the lower rim of the foramen. There is no well marked suture dividing the reduplication of the sternum from the propodeum, but the manubrium of the endoskeleton ends some distance from this foramen and it probably marks the limit of the metasternum. The integument of the propodeum is thrown

into external folds of fairly constant character. Those near the coxal articulations are more subject to variations. The whole metathorax of *Monodontomerus* may be characterized by the abundance of such folds. In the figures these folds have been represented by heavy lines.

THE ENDOSKELETON

The endoskeleton of the metathorax consists of a pair of diverging furcal arms (Figs. 19, 39, 40, 41, AFU), arising near the anterior edge of the sternum without leaving a noticeable pit. Each arm expands into a plate which fuses at the sides with the large pleural ridge. The whole anterior edge of the metapleuron (INP) is inflected for a considerable distance. Dorsally this inflection joins with the pleural ridge. Behind the pleural arms a low manubrium (MFU) arises from the sternum and its reduplication. Externally no groove is apparent along this line though a greater density of the chitin shows externally as a line.

The Legs

The legs are, in general, of the cursorial type, although the metathoracic legs are greatly developed in connection with the habit of jumping into the air prior to flight. Detailed description of the legs seems unnecessary and the reader is referred to the figures (Figs. 42, 43, 44, 48, 49, 50). The tarsi (TAR) are five-jointed and bear a terminal pretarsus (PTAR). Ventrally they have large movable setae. The tibiae of the prothoracic and mesothoracic legs bear a single spur or calcar (CC) while the metathoracic tibia (TB) bears two such spurs. The latter also possesses a number of short spines dorsally and terminally. The mesofemur (FM) is divided proximally by a definite suture at which there is no movement. The sclerite thus set off is characteristic of many parasitic Hymenoptera and is called the second trochanter (2TR). The profemur shows similar incomplete division but no evidence of this can be detected in the metafemur. The metafemur bears a distal toothlike projection (DF). Similar but less definite teeth are formed on the procoxa and metacoxa by a flaring of the integument. The coxae (CX) are comparatively large and cover most of the thorax ventrally (Fig. 24).

The Articulations

The articulations of the legs follow the general insect form. The procoxa (Figs. 21, 51) articulates with the inner angle of the episternum without the aid of a specialized condylar apparatus. The meso- and metacoxal articulations (Figs. 52, 53) are monocondylic, a process developed at the end of the pleural suture articulating with a shallow socket on the coxa. Thus all three coxae, having a single articulation with the pleuron, are not limited to uniplanar movement. They may be observed to move in a rotary manner. The coxotrochanteral joint (Figs. 54, 55, 56) in all three legs is dicondylic though one condyle is poorly developed in the prothoracic leg. There is a certain flexibility throughout at this joint. The trochanterofemoral joint (Figs. 54, 55, 56) of all the legs is without any definite condylar articulations. The movement is very restricted at this joint. The femur and tibia articulate

by a hinge joint or ginglymus allowing movement only in one plane (Figs. 57, 58, 59). The tibiotarsal articulations are monocondylic (Figs. 60, 61, 62, 63).

The Pretarsus

The pretarsus (Figs. 42, 43, 44) is similar in all three legs and corresponds closely to that found in other insects. It consists essentially of a pair of large lateral claws, the ungues (UN), and a median fleshy lobe, the arolium (AR). The claws are bilobed having a long curved upper point and a wider basal lobe. They articulate dorsally with the last tarsomere, which is not differentiated into a distinct unguifer. The arolium is supported dorsally by a flask-shaped orbicula (OR) and ventrally by the calcanea or unguitractor (UNG). An incomplete ringlike sclerite, the camera (CA), supports the arolium in a more terminal position.

The Wings

The wings of *Monodontomerus* (Figs. 65, 66) are similar to those of other chalcids in having reduced venation, and a condensation of the bases of the veins to form scales or sclerites for articulation with the axillary wing sclerites. The front wing is much larger than the hind one, a feature to be expected since the flight muscles are mainly concentrated in the mesothorax. Both wings work together, however, a frenulum (Figs. 64, 66, FL) of the hind wing catching a stout rib (Fig. 65, WR) on the posterior margin of the front wing. The wings are covered with setae or macrotrichia except for the basal areas which are comparatively naked. When not in use the wings are folded flatly over the back and extend slightly beyond the tip of the abdomen.

Burks (2), who has made a study of chalcidoid venation, considers the submarginal vein (Figs. 65, 66, SMV) to be composed of subcosta and radius (SC + R), and the marginal and postmarginal veins (MV, PMV) to be the first radial branch (R₁). The stigmal vein (STV) is the second radial crossvein R₂. Rows of macrotrichia trace the paths of the obsolete median and cubitus vein (M + CU) and its branches (M, CU'). The hind wing shows only the submarginal vein.

The Wing Articulations

Each wing is attached to the body by a membranous basal area containing a number of small articular sclerites, the pteralia or axillaries. These are very minute in *Monodontomerus*, particularly in the hind wing.

In the forewing the first axillary sclerite (Fig. 67, AX1) is L-shaped. It articulates with the anterior notal wing process of the mesothorax, the edge of the alar plate and the large subcostal scale (SCS) embracing the wing base. The second axillary (Figs. 67, 69, AX2) presents both a dorsal and a ventral sclerotization in the wing base. Its ventral area lies on the pleural wing process and the dorsal edge of the epimeron, while dorsally it articulates with the first axillary and the basal scale of the radius. The third axillary (AX3) articulates with the posterior wing process, the second axillary, and the basal scale representing the condensed anal veins. Ventrally a basalare (BAL)

articulates with the anterior surface of the pleural wing process and with the subcostal scale. The posterior margin of the articular membrane is not thickened into an axillary cord.

In the hind wing the sclerites are similar but much smaller. The first axillary (Figs. 68, 69) articulates with the anterior notal wing process, the edge of the alar plate and the base of the submarginal vein. The second axillary articulates with the pleural wing process, the first axillary and the base of the submarginal vein. The third axillary articulates with the posterior notal wing process, the second axillary, the thickened edge of the wing, probably representing the anal veins, and a small sclerite (M'), which may be the condensed base of the median vein. A basalare joins the submarginal base to the pleural wing process. There is no definite axillary cord.

The Abdomen

The abdomen of *Monodontomerus* (Figs. 70, 71, 72, 73, 74, 75) consists of 10 segments, though much modification has occurred, resulting in a partial or complete disappearance of some of the segments as such. The first abdominal segment has become incorporated into the thorax as the propodeum. The eighth and ninth segments are modified in connection with the development of the external genitalia; they exhibit the most essential difference between the male and female insects. There is such an extraordinary telescoping of the segments that most of the area of the various sclerites is hidden.

THE FEMALE ABDOMEN

The abdomen of *Monodontomerus* appears to be sessile owing to the small development of the petiole, the second abdominal segment (Figs. 35, 36, 37, 70, 72, 73, 2T, 2S). The petiole is a small ringlike segment, embraced by the propodeal foramen in front and articulating with the third segment behind. Internally it bears a transverse chitinous tendon (Fig. 37) which divides it into an upper and a lower portion, probably representing the fused tergum and sternum. The sternum is very slender ventrally but flares backwards at the sides. The walls are dense and rugulose. Through the lumen pass the alimentary canal, the aorta, the nerve cord, and the longitudinal tracheal trunks. The third segment is large, particularly the sternal portion (3S), which is bent into a V-shaped trough along the median line. At the anterior end of the "V" the sternum projects downward forming a distinct lip. The posterior border is without a median indentation so characteristic of many chalcids. At each side of the third abdominal sternum there is a raised portion for articulation with the tergum.

Segments 3, 4, 5, 6, and 7 are all complete. The terga are large and come down the side almost hiding the sterna from a lateral view. Each sternum bears a pair of internal ridges or apodemes for the attachment of intersegmental muscles. The seventh sternum is particularly well developed and has lightly sclerotized lateral portions projecting posteriorly, giving it a Λ -like appearance. The ovipositor issues beyond the seventh sternum.

The eighth segment is represented by a small dorsal tergum and two lateral, lightly chitinized plates projecting forward, the latter being hidden by the terga in front (Figs. 93, 99, 100, 8S). These lateral sclerites are probably the divided eighth sternum. The eighth tergum contains a pair of spiracles (Fig. 99, SP3). The ninth segment is represented by the inner and outer plates of the ovipositor. The inner plates are considered to be the ninth sternum and the outer plates the ninth tergum. This tergum carries a pair of small appendages, bearing five sensory hairs of which one is considerably larger than the rest. James calls these sensory plates (SPL). They have been erroneously called cerci. The inner plates end in a pair of large sensory palps (PAL), covered with setae and enveloping the sting. The female uses these in investigating the host cocoon before oviposition.

The anal papilla (ALP) represents the 10th segment and any segments posteriorly. Dorsally it is hardened, forming a small plate, typically bearing two setae on each side. Often this plate is not symmetrical, owing to an underdevelopment of one side.

THE MALE ABDOMEN

The abdomen of the male (Figs. 71, 74, 75) differs from that of the female principally in size and in the modification of the terminal segments. The eighth segment is complete in the male, being formed of a tergal and a sternal plate. The ninth tergum bears a pair of sensory plates similar to those of the female. The ninth sternum is divided into two halves separated by a narrow longitudinal membrane. The aedeagus (AED) issues from the abdomen beyond the ninth segment. The 10th segment is represented by a small anal papilla. Ventral to the papilla and running forward to join it to the aedeagus sheath (AES) is a narrow lightly sclerotized plate (Fig. 85, VI), which James (7) believes to represent the "valva interna" of Zander. This may be part of the 10th segment.

The Female Reproductive System

The generative organs of the female consist of a pair of ovaries, each possessing an oviduct which unites with its fellow to form a common oviduct and vagina. There are two pairs of accessory glands and a spermatheca or receptaculum seminis, which also possesses a pair of glands.

THE OVARIES

The ovaries (Fig. 83) are long in comparison to the length of the abdomen and thus must be convoluted. They pass backwards from the oviducts, curve upwards in the region of the sixth notum, and pass forwards to the front of the abdomen, then curve downwards and backwards to the ventrolateral regions of the crop, from which they ascend to the dorsolateral regions of the latter. Here the vitellarium changes to germarium and the latter is produced backwards on the dorsolateral surface of the crop.

Each ovary consists of three ovarioles (OVL) which adhere closely, *in situ*, by connective tissue strands. Each ovariole may be divided into two main regions, the germarium and the vitellarium.

The germarium (GER) is the terminal portion of the ovariole and contains undifferentiated cells which are transformed into egg cells and nutritive cells. The germarium adheres to the dorsolateral surface of the crop. No terminal filament could be identified.

The vitellarium (VIT) includes all the remainder of the ovariole and contains eggs in all stages of development. Distally in the vitellarium the eggs (EG) are small, surrounded by the follicular epithelium (FE), and alternate with the nutritive or nurse cells (NC). This polytrophic type of ovariole is characteristic of Hymenoptera in general. Proximally, the nutritive cells have degenerated, a chorion (CH) has been produced by the follicular epithelium, and, at the entrance to the oviduct, there are several mature eggs lying free in the ovarioles. The eggs are banana-shaped, having a short pedicel at one end. The chorion is produced externally into numerous short spines which probably represent the intercellular spaces in the follicular epithelium. Each ovariole of a newly emerged female contains three or four mature or nearly mature eggs, so that about 20 or 25 eggs are ready for oviposition soon after emergence.

THE OVIDUCTS

The three ovarioles (OVL) on each side unite to form an oviduct (OV) which passes forwards and meets its fellow of the opposite side, forming a short median tube, the common oviduct (COV). Two pairs of glands open into the common oviduct. James (7) has named these the primary and secondary accessory glands (1AG and 2AG). The duct of the spermatheca also opens into the common oviduct.

THE SPERMATHECA

The spermatheca or receptaculum seminis (SPM) consists of a short duct, expanded at its end to form a cavity for the storage of sperms and emptying into the common oviduct. Two accessory glands (AGS) are associated with the spermatheca.

THE VAGINA

The common oviduct opens into the vagina (VAG), which passes forward to the base of the sting and overlies the poison sac. The dorsal wall bears a sclerite (Figs. 81, 83, VSC) which becomes very thin at the edges, the latter being produced downwards at the side. This sclerite serves as an insertion for muscles attaching the vagina to the seventh abdominal sternite. The vagina bends downwards around the base of the ovipositor and opens in the middle line, just in front of the base of the seventh sternite to form the female genital opening. This end-portion of the vagina receives the aedeagus during copulation and therefore may be called the bursa copulatrix.

THE FEMALE GENITAL ARMATURE

The genital armature of the female is composed, as in other insects, of three pairs of gonapophyses, developed from the eighth and ninth abdominal segments. In *Monodontomerus* these gonapophyses are greatly specialized to form the ovipositor, an organ for the transference of eggs from the genital aperture to a position within the host cocoon. The ovipositor is the best investigated organ of chalcidoid anatomy. Imms (6), James (7), and Hanna (3) have made studies which agree, both with each other, and essentially with Snodgrass's investigation in the honey bee.

The essential structure of the ovipositor is a long egg-tube formed by the co-operation of paired stylets and stylet sheaths.

The Stylet Sheaths

The stylet sheaths (Figs. 76, 77, 78, 79, 80, 82, STYS) are a pair of long chitinous rods which fuse at the apex and bear toothlike projections, which act as saw-teeth for drilling into the host cocoon (Fig. 79). At its base each sheath expands into a large heavily sclerotized knob, the rotatory process (RP). The paired rotatory processes are connected with one another by a series of transverse chitinous ribs (RPR). The stylet sheaths pass upwards and backwards from the rotatory processes as a pair of diverging arms.

The Stylets

The stylets (Figs. 76, 77, 78, 80, 82, STY) are a pair of long hollow chitinous rods which become pointed and bladelike at the apex. Together with the stylet sheaths they form the essential boring apparatus and egg tube (Fig. 80, ET) of the ovipositor. Laterally each stylet bears a longitudinal groove into which a ridge of the sheath fits. This helps to hold the component parts of the sting in juxtaposition. Anteriorly the stylets diverge from one another but remain in contact with their corresponding sheath. James believes that the poison fluid passes down the stylet canals and enters the wound by one or two fine pores near the apex. Such pores were not seen in *Monodontomerus*.

For the proper functioning of the ovipositor three other components are necessary, the inner plates, the outer plates, and the fulcral plates.

The Inner Plates

The inner plates (Figs. 76, 77, 78, 82, IP) of Imms (6), corresponding to the oblong plates which Snodgrass found in the bee, consist of a pair of long lamellae, diverging and expanding proximally to fuse with the diverging arms of the stylet sheaths. They are produced forward lateral to the rotatory processes as the pivoting sclerite (IPP). Throughout most of its length, each plate bears an inner longitudinal rib (IPR), which contributes to the rigidity. Proximally this rib articulates with the fulcral plate. The plates are joined ventrally by a membranous connection which bulges upwards forming a groove to receive the sting. Near the apex, the outer edges of the plates curl over and join in the mid line forming a short bridge (IPB). Terminally the inner plates bear a pair of sensory palps (PAL) which enclose the sting when at rest.

The Outer Plates

The outer plates of Imms (Figs. 76, 77, 78, 82, OP), corresponding to the quadrate plates of Snodgrass, occupy a lateral position enclosing the inner plates. The dorsal edges are reflected inward, and distally they meet forming the ninth tergum. This tergite bears a pair of sensory appendages with five hairs (SPL). Proximally the outer plates articulate with the fulcral plates. They are strengthened by chitinous ribs (OPR).

The Fulcral Plates

The fulcral plates of Imms (Figs. 76, 78, 82, FP) correspond to the triangular plates of Snodgrass. Each is a narrow sclerite, fused dorsally with the corresponding stylet and articulating with the inner and outer plates.

The various parts of the ovipositor may be homologized with those of generalized insects, there being agreement among the various investigators. The stylets are formed from the gonapophyses of the eighth segment and correspond to the ventral valvulae of orthopterous insects. The fulcral plate is also developed from the eighth sternum and probably corresponds to the first valvifer. The inner gonapophyses of the ninth segment produce the stylet sheaths, which consequently correspond to the inner valvulae of the Orthoptera. The inner plates are developed from the outer gonapophyses of the ninth segment and thus are homologous with the dorsal valvulae or lateral gonapophyses of other insects. The proximal expansions of the inner plates, which fuse with the diverging arms of the stylet sheaths, seem to correspond to the second valvifer. The outer plates, as can readily be seen, are formed from the ninth tergum and distally retain their dorsal connection.

For a discussion of the muscles of the ovipositor and the method of functioning, the reader is referred to James (7), Hanna (3), and Imms (6).

THE POISON APPARATUS

The poison apparatus is well developed and consists of two glands (Fig. 84) which correspond to the acid and alkaline glands of the bee.

The acid gland (ACG) is a long tubular structure lying in the floor of the ovipositor. It has a narrow lumen surrounded by large, columnar, darkly staining cells with prominent nuclei. Anteriorly it opens into a capacious reservoir (ACR), situated between the diverging arms of the sting and beneath the vagina. Its contents coagulate on coming in contact with alcohol. A small duct leads from the reservoir to the base of the sting.

The alkaline gland (ALG) is an elongated structure, overlying the acid gland reservoir and opening into the base of the sting by a fine long duct.

The Male Reproductive System

In the male the reproductive system consists of the paired testes, vasa deferentia, vesiculae seminalis, glandulae mucosae, an unpaired ductus ejaculatorius, and the genital armature.

THE TESTES

The testes (Fig. 85, TT) are a pair of small sacs, lying above the mesenteron on each side at about the level of the fifth or sixth abdominal tergites. They attain their maximum size in the pupa, since after eclosion of the adult spermatogenesis is largely completed and the testes tend to shrink in size.

THE VASA DEFERENTIA

These are two thick walled tubes (VD), leading from the testes downwards to the vesiculae seminales.

THE VESICULAE SEMINALES

The seminal vesicles (VSM) are morphologically dilations of the walls of the vasa deferentia for the storage of sperm.

THE GLANDULAE MUCOSAE

These are bean-shaped mucous glands (GM) lying lateral to the seminal vesicles which open into them.

THE DUCTUS EJACULATORIUS

The ejaculatory duct (DE) is a median unpaired tube, formed by the union of the two efferent ducts from the mucous glands. It enters the aedeagus sheath and continues posteriorly between the aedeagus arms to the genital aperture at the tip of the aedeagus. Between the aedeagus arms and ventral to the ejaculatory duct is a long tubular gland (AEG), opening into the latter. Anteriorly it stretches beyond the aedeagus sheath.

THE MALE GENITAL ARMATURE

The male genitalia consist of the aedeagus with its sheath, situated in an invaginated chamber of the ninth segment.

The aedeagus sheath (Figs. 85, 87, 88, AES) consists of an incomplete chitinous cylinder, a little flattened dorsoventrally, lying in the middle line on the floor of the abdomen. Dorsally the sheath is made into a complete cylinder by a long, subtriangular, thin, chitinous plate (VI), extending from the anterodorsal end of the sheath to the end of the abdomen beneath the anal papilla. At its posteroventral extremity the sheath bears a pair of small processes, ending in three short curved spines. These so-called claspers (CL) probably aid in keeping the aedeagus within the bursa of the female.

The aedeagus (Figs. 85, 86, 87, 88, AED) is a flattened hollow structure, pointed apically and extending backwards on each side in a long chitinous arm (AEDA). It is traversed by the ductus ejaculatorius which opens by a subapical pore (GP). A number of minute papillae can be seen near the tip on each side of the aedeagus (Fig. 87). The aedeagus can be protruded from its sheath for about half its length.

The Digestive System

The alimentary canal in *Monodontomerus*, as in other insects, can be divided into three main regions, the stomodaeum, the mesenteron, and the proctodaeum.

THE STOMODAEUM

The stomodaeum (Figs. 93, 106) includes that part of the alimentary canal which arose by an invagination of the ectoderm from the anterior end of the body, and thus is lined by a chitinous intima. It may be divided into preoral cavity, pharynx, oesophagus, crop, and proventriculus. The wall of the gut in this region consists of an inner chitinous intima (IN), a layer of flat epithelium (ETH), bounded by a basement membrane (BM), and a layer of inner longitudinal and outer circular muscles (LM, CM).

The Preoral Cavity

The preoral cavity (Fig. 106, POR), often erroneously called the buccal cavity, lies below the true mouth opening and is bounded by the mandibles, the labrum, and the labium. Functionally it serves as a mouth. The true mouth lies between the epipharynx and the hypopharynx and leads into the pharynx. The epipharynx bears short hairs called spicules by Snodgrass (11) which project into the mouth.

The Pharynx

The pharynx (Figs. 93, 94, 95, 106, PHY) lies in the anterior part of the head close behind the clypeus and frons, extending dorsally from the mouth to above the level of the antennal sockets, where it turns posteriorly and contracts to the narrow oesophagus. In the floor of the pharynx there is a wide chitinous pharyngeal plate (Figs. 93, 94, PHP) which dorsally is prolonged into two arms, curving upwards in the lateral walls of the pharynx. The intima (Fig. 95) is very thick, and on the anterior wall bears a number of spicules projecting downwards. The epithelium is composed of rather small cuboid cells bounded by a definite basement membrane. The pharynx is well supplied with both dilator and constrictor muscles (Fig. 106, DM, COM) which make it an efficient sucking apparatus.

The Oesophagus

The oesophagus (Figs. 93, 94, 106, OE), a narrow thin-walled tube, passes from the pharynx, between the great cephalic nerve masses and above the body of the tentorium, to the thorax which it traverses to enter the abdomen where it expands into the crop. The intima is thin and the epithelium appears as a few scattered nuclei beneath it. Muscle fibers could not be seen.

The Ingluvies

Owing to the highly developed thoracic musculature, the storage and digestive sections of the gut are located in the abdomen. The ingluvies or crop (Figs. 91, 93, CR) is the chief storage chamber and is formed by the expansion of the oesophagus immediately on passing through the petiole.

The wall of the crop is thin, translucent, and highly distensible. The wall is formed of a layer of thin intima (Fig. 91), which is thrown into folds if the organ is not completely distended, an epithelium revealed only by scattered nuclei, an inner layer of occasional longitudinal muscle fibers, and a well developed outer layer of circular muscles.

The Proventriculus

The crop passes into a short narrow very muscular portion of the gut, the proventriculus (Figs. 92, 93, PVP), which controls the passage of food from the stomodaeum into the mesenteron. The proventriculus projects into the crop as a low mound, often called the calyx, and into the mesenteron a considerably greater distance, forming the stomodaeal or cardiac valve. The proventriculus is triangular in transverse section and the lumen is invaded by three folds or lips, by means of which it can be closed off.

The wall of the proventriculus is thick. A heavy intima (Fig. 92) bearing spicules at the anterior end lines the organ. Only the nuclei of the epithelium can be seen. The muscle layer is well developed and consists of an inner and outer layer of longitudinal muscles enclosing a middle layer of circular muscles.

THE MESENTERON

The mesenteron is the chief digestive and absorptive portion of the gut. It consists of a large stomach or ventriculus (Figs. 93, 96, VT) lying above the ovipositor in segments 6, 7, and 8 of the abdomen. Since it is the endodermal portion of the gut it lacks a chitinous intima. The epithelial cells are large columnar subhexagonal cells, limited by a basement membrane. They have large nuclei which tend to take up a position towards the base of the cells. The cytoplasm is granular and stains deeply. No trace of a peritrophic membrane could be found. There is a weak layer of circular muscles but longitudinal muscles were not seen.

THE PROCTODAEUM

The proctodaeum is the posterior part of the alimentary canal, formed by an ectodermal invagination and consequently lined by a chitinous intima. It consists of the intestine and the rectum.

The Intestine

The mesenteron narrows abruptly to form a poorly marked portion of the intestine, the pylorus, into which the Malpighian tubules open and which, in some insects, is the seat of a well-developed pyloric valve. The intestine (Figs. 93, 98, INT) proceeds forward a short distance and then turns backwards to join the rectum.

The intestine is lined by a thin intima (Fig. 98). The epithelial cells are subcuboid with large nuclei and darkly staining cytoplasm. Circular muscles only could be seen.

The Malpighian tubules (Fig. 93, MAL) vary in number from 16 to 20 in the female and 14 to 16 in the male. In cross section two or three cells can be seen bordering a narrow lumen (Fig. 97).

The Rectum

The intestine expands abruptly to form the rectum (Fig. 93, RC) which in turn leads to the anus in the eversible anal papilla (ALP). The wall of the rectum is very thin and so transparent that the contents can be clearly seen. It consists of a fine intima beneath which appear scattered nuclei of the epithelium. A poorly defined layer without evident striation, but which the author has taken to be the muscular layer, is external to this.

On either side of the opening of the intestine a large convex body is seen in the rectal wall, projecting well into the lumen. These are the rectal glands (RGL). Their structure is difficult to make out. They seem to be composed of a thick inner wall and a thin outer wall, enclosing a narrow lumen. The inner wall is composed of large pyramidal radially-arranged cells with large nuclei and indistinct cell boundaries and is covered with a thin intima where it projects into the rectal lumen. The outer wall is thin and made up of small cells.

THE SALIVARY GLANDS

As many as 10 different kinds of salivary glands have been described in Hymenoptera, though they are not all present in the same species. In *Monodontomerus* only two kinds were found, the thoracic and sublingual.

The thoracic or labial salivary glands (Fig. 93, LGL) are the best developed in Hymenoptera. In *Monodontomerus* they consist of a pair of elongated cylindrical glands lying in the prothorax, each equipped with a duct (LD) which passes forward to the body of the tentorium, where it joins with its fellow to form a common duct passing below the corporotentorium to empty, at the base of the hypopharynx, in a groove, the salivarium (Fig. 106). The duct is stout walled and possesses a chitinous intima which is thickened at regular intervals to form threadlike ridges, projecting into the lumen and resembling the taenidia of tracheae (Fig. 90).

The sublingual glands (Figs. 89, 106, HGL) consist of a number of large cells lying in the hypopharynx and opening by a duct in its floor. In *Harmolita* James (7) has found mandibular glands near the base of each mandible. Pharyngeal glands along the posterior wall of the pharynx have been described in some Hymenoptera. In *Monodontomerus* there are in these positions large cells which are possibly fat cells, especially since no ducts were found in connection with them.

The Respiratory System

Since the comparative anatomy of the respiratory system in the Hymenoptera particularly in the chalcids has been neglected and the terminology is sparse, the various tracheae will be referred to by number rather than by a name which further research might show to be unsuitable.

The tracheal system (Fig. 99) consists essentially of two longitudinal trunks, running from the cervical region to the eighth abdominal segment. The largest tracheae in the body (1) are found in the mesothorax, lying on either side of the middle line and connected anteriorly by the middle connective (CMD). They separate and pass laterally to receive the stigmatic branch (ST1) from the first spiracle (SP1). The trunks then traverse the prothorax where they are joined by the anterior connective (CAT), but they proceed as separate trunks to the head, where each breaks up into a dorsal set of branches and a ventral set of branches. The dorsal branches (2, 3, 4) supply the dorsal region of the head and brain. The ventral branches (5, 6, 7) supply the ventral region of the head and brain, while a trachea to each antenna (8) usually arises from the base of 6.

In the mesothorax there is a pair of lateral trunks (9), lying just inside the pleural wall near the tergopleural junction. They give large branches to the tergosternal muscles and small branches (10, 11) to the first and second wings, respectively. Each joins trachea 1, both anteriorly and posteriorly, and thus forms a ring surrounding the tergosternal muscles. A large dorsal trunk (12) joins 1 and 9 at each end of the ring. The resultant pair of trunks curve upwards and redivide many times to supply the dorsal wing muscles. This pair is joined by a dorsal connective (CDO). A stigmatic branch (ST2) from the propodeal spiracle (SP2) joins the common chamber formed by tracheae 1, 9, and 12. From this chamber a large trunk (13) passes backwards and narrows to enter the petiole and abdomen where it is continued as the main longitudinal trunk of the abdomen (TLA).

The prothoracic leg is supplied by three tracheae. One (14) passes forward from 1 to the prothorax, where it gives branches to the ganglion and then curves posteriorly beneath the furca to enter the coxa. Another (15) arises from the longitudinal trunk in the prothorax near the anterior connective, while a third (16) comes off the anterior common chamber formed by the union of tracheae 1, 9, and 12.

The mesothoracic leg is supplied by a large tracheal branch (17) and a smaller one (18) from 1.

The metathoracic leg is aerated by two branches (19, 20) from 13, and a branch (21) from 9.

In the abdomen tracheae 13 are continued as the main longitudinal trunks (TLA), lying in a dorsolateral position and opening by a stigmatic trunk (ST3) and spiracle (SP3) on the eighth tergum. They give rise to four pairs of highly branched tracheae which aerate the ovaries and fat body in the female and the fat body in the male. From the base of the longitudinal trunks in the female (Fig. 99) and from the stigmatic branch in the male (Fig. 102), a lateral longitudinal branch (22) proceeds forwards supplying the ovaries and fat body, while a posterior branch (23) supplies the terminal portion of the abdomen and rectum.

The paired longitudinal trunks are joined by a posterior connective (CPT) from which a number of tracheae arise. This region is much better developed

in the female and is subject to considerable variation, even on the two sides of the same individual. In the male (Fig. 102) the branches are small and supply the aedeagus. In the female (Fig. 99) the branches are much larger. A branch (24) proceeds forward and aerates the ventral wall of the vagina and probably the poison sacs and adjacent structures. Another branch (25) supplies the common oviduct, accessory glands, and spermatheca and also the abdominal ganglia. Most of the other branches (26) enter the muscles of the ovipositor. A minute trachea (27) passes backwards on the floor of the ovipositor valves and supplies the terminal portions of the latter.

The adult *Monodontomerus* has only three pairs of spiracles though more are present in the larva. The first spiracle is situated in the membrane between the pro- and mesothorax (Figs. 17, 99, SP1). Morphologically it belongs to the mesothorax. The second spiracle (Figs. 23, 38, 99, SP2) opens on the propodeum but belongs to the first abdominal segment. The metathoracic spiracle is absent in the adult of *Monodontomerus*. The third spiracle (Figs. 99, 102, SP3) opens on the eighth abdominal segment, to which it belongs. The spiracles usually present on the second to seventh abdominal segments of many insects have disappeared in this form.

The Central Nervous System

The central nervous system (Figs. 100, 102) consists of two great cephalic nerve masses, one above and one below the alimentary canal, forming the cephalic component; three thoracic ganglia, connected by an apparently unpaired nerve cord, forming the thoracic part; and an abdominal component, formed of two ganglia in the female and one in the male.

THE CEPHALIC NERVE CENTERS

The cephalic nerve centers (Figs. 100, 101, 103, 104, 105, 106, 107) consist of a supraoesophageal ganglion (GDO) or brain, lying above the oesophagus, a suboesophageal ganglion (GVO), lying below it and joined to the former by short thick connectives lateral to the oesophagus. These centers are large and occupy most of the space in the head. The brain is divisible into three regions the protocerebrum, the deutocerebrum, and the tritocerebrum.

The Protocerebrum

The protocerebrum (Figs. 103, 105, 107, 1BR) is composed of a pair of protocerebral lobes interconnected by a median commissural system, the central body (Fig. 103, CB). On each side the protocerebrum is produced into the optic lobes (Figs. 101, 103, 104, OPL), supplying the compound eyes. In section each optic lobe or tract is seen to be formed of three zones, an outer perioptic (POPT), connected to the ommatidia by a layer of postretinal fibers, a middle epiptic (EOPT), and an inner opticon (OPT).

The paired mushroom bodies or corpora pedunculata (Fig. 103, CP) are buried dorsally in the cortical layer of the protocerebrum. Each consists of two concave discs or caps, forming the calyx (CYX), supported on a bifurcated

stalk or cauliculus, arising ventral to the central body. The concave surface of one cap points posteriorly, that of the other points dorsolaterally; both are filled with darkly stained cortical cells. The pedicel of the mushroom bodies was not seen.

Dorsally, between the corpora pedunculata, are the ocellar lobes which give rise to the ocellar nerves (Figs. 101, 103, 105, 107, NO) supplying the ocelli. The median nerve appears double in section.

The Deutocerebrum

The deutocerebrum (Figs. 104, 105, 107, 2BR) lies in a position antero-ventral to the protocerebrum. In section it has a fasciculated appearance. It consists of a pair of antennary or olfactory lobes which give rise to the antennary nerves (Fig. 101, NA).

The Tritocerebrum

The tritocerebrum (Fig. 105, 3BR) is hidden behind the deutocerebrum so that it is visible only in sections. It consists of a pair of minute lobes giving off a pair of frontal commissures (Figs. 101, 105, NF), connecting it to the frontal ganglion (Fig. 106, GF).

The Suboesophageal Ganglion

The suboesophageal ganglion (Figs. 101, 103, 106, 107, GVO) lies beneath the oesophagus and is joined to the brain by a pair of stout thick commissures. Posteriorly it is connected to the prothoracic ganglion by the ventral nerve cord (Figs. 100, 106, NV). It gives rise to three main nerves supplying the mandibles (Fig. 107, NMD), maxillae (NMX), and labium (NL).

The insect head has been generally regarded as formed of six segments, the three divisions of the brain and triple nature of the suboesophageal ganglion being considered as proof of this. Sollaud and Snodgrass have advanced the theory that the arthropod head is formed from the annelid prostomium plus the first four somites of the body. Thus the protocerebrum and deutocerebrum, supplying the eyes and antennae, are taken to be secondary divisions of the prostomial ganglion; the tritocerebrum to be the ganglion of the first true somite, and the suboesophageal ganglion the coalesced nerve centers of the second, third, and fourth somites, bearing respectively the mandibles, maxillae, and labium.

THE THORACIC NERVE CENTERS

The thoracic nerve centers (Fig. 100) are three in number, one to each segment, and are connected by the ventral nerve cord. Both nerve cord and ganglia show evidence of their double nature in section. The meso- and metathoracic ganglia show incipient signs of a coalescence already accomplished in the bee and the wasp.

The Prothoracic Ganglion

The prothoracic ganglion (GT1) lies on the floor of the prosternum and is supported by the profurca. It gives off a nerve to the prothoracic muscles

and a large one to the leg (NL1), from which a small branch is given off to the muscles of the coxa.

The Mesothoracic Ganglion

The mesothoracic ganglion (GT2) lies in the hind part of the mesothorax, supported on the furca. It is considerably elongated forwards and joins the prothoracic ganglion by a long connective. Between the prothoracic and mesothoracic ganglia, at the level of the prepectus, there is a swelling on the nerve cord which James (7) has termed the mesothoracic accessory ganglion (GAC). It gives rise to two large pairs of nerves. The first pair (NW1) lie close upon the epidermis and pass to the mesothoracic wing giving off branches to the tergosternal muscles on the way. The second pair supply the dorsal longitudinal muscles (ND). The mesothoracic ganglion gives rise to a pair of nerves (NM), supplying the direct wing muscles and probably also sending branches to the indirect wing muscles. The largest nerves are those supplying the legs (NL2). Where these are given off from the ganglion, a nerve supplying the coxal muscles arises.

The Metathoracic Ganglion

The metathoracic ganglion (GT3) lies in the anterior part of the metathorax and propodeum, supported on the metafurca. It consists, at least, of the fused ganglia representing the metathorax and the first two abdominal ganglia, which latter supply the propodeum and the petiole. Its largest nerves are those supplying the hind legs (NL3). Small proximal branches of these supply the coxal muscles. It also bears a small nerve, supplying the hind wing (NW2), and at least two other nerves, supplying the muscles of the propodeum and metathorax. Nerves to the petiole were not found but they would be extremely small.

THE ABDOMINAL NERVE CENTERS

In the female there are two abdominal ganglia connected by a very short commissure and lying dorsal to the common oviduct. The first ganglion (Fig. 100, GA1) lies right upon the common oviduct, the second (GA2) is subspherical and lies posterior to the common oviduct between the oviducts of each side. There is a long nerve cord, connecting the ganglia to those in the thorax. A nerve to the third abdominal segment arises from this connective. The first abdominal ganglion gives off four pairs of visible nerves to segments four, five, six, and seven and thus seems to represent the four fused ganglia of these segments. The second abdominal ganglion supplies the ovipositor and terminal segments of the abdomen and probably represents the last ganglionic mass of the larval nerve chain. Three pairs of nerves arise ventrally and pass forwards to supply the ovipositor and floor of the abdomen. Three pairs pass backwards. One pair (NAT) supplies the terminal floor of the abdomen and the rectum (NR), the others supply the eighth and ninth segments.

In the male there is one central nerve mass (Fig. 102, GA1), situated on the third sternum and probably representing the last six ganglia of the larval

abdomen. It gives off paired nerves to segments three, four, five, six, and seven. It is continued posteriorly as a median nerve, which proceeds backwards to the level of the aedeagus sheath where it divides into two branches, one passing to each side of the sheath and giving off three nerves to the genital armature. Each main branch continues backwards to supply the terminal portion of the abdomen. Proximally the median nerve is slightly swollen. Just before it divides, the median nerve gives off two pairs of small nerves, the first of which supplies the glandulae mucosae.

Thus there is considerable difference between the abdominal nervous system of the male and female. At first sight it appears that more cephalization has occurred in the male, since there is only one definite nerve center in an anterior position, while the female possesses two such centers in a more posterior position. The author believes that further inspection casts doubt on this view. The posterior position of the female nerve centers is necessary, since the upwardly diverging arms of the ovipositor occupy practically all the space in the anterior part of the abdomen. In the female the nerve to the third abdominal segment arises from the ventral nerve cord, connecting the abdominal with the thoracic nerve centers, and presumably its tracts end in the compound metathoracic ganglion. In the male this nerve arises from the abdominal ganglion. Thus the third thoracic nerve mass in the female consists of the metathoracic ganglion fused with those of the first three abdominal segments and thus exhibits more cephalization than the nerve mass in the male, which consists of the metathoracic ganglion and two abdominal ganglia. In the female the first abdominal ganglion gives rise to four paired nerves supplying segments four to seven, while that of the male supplies the same segments as well as segment three. The second abdominal ganglion, in the female, supplies the eighth segment and those posterior and innervates the reproductive system and genital armature. It is the author's opinion that this ganglion can be homologized with the median nerve and its swelling in the male, since these also supply the eighth and terminal segments and the reproductive system.

THE VISCERAL NERVOUS SYSTEM

The stomatogastric component of the visceral nervous system consists of a frontal ganglion (Fig. 106, GF) on the anterior surface of the pharynx, connected to the tritocerebral lobes by the frontal commissures (Fig. 105, NF), and a recurrent nerve (NRE) passing backwards on the dorsal surface of the oesophagus to the hypocerebral ganglion just behind the brain.

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References

1. BUGNION, E. Recherches sur le développement postembryonnaire, l'anatomie et les mœurs de l'*Encyrtus fuscicollis*. Rec. Zool. Suisse : 435-534. 1890.
2. BURKS, B. D. A study of chalcidoid wings. Ann. Entomol. Soc. Am. 31 : 157-160. 1938.
3. HANNA, A. D. The male and female genitalia and the biology of *Euchalcidia caryobori* Hanna (Hymenoptera, Chalcidinae). Trans. Roy. Entomol. Soc. London, 82 : 107-136. 1934.
4. HANNA, A. D. The morphology and anatomy of *Euchalcidia caryobori* Hanna. (Hym. Chalcidinae). Bull. soc. entomol. Egypte, Le Caire, 19 : 326-361. 1935.
5. IMMS, A. D. Observations on the insect parasites of some Coccidae. I. On *Aphelinus mytilaspidis* Le Baron, a chalcid parasite of the Mussel scale (*Lepidosaphes ulmi* L.). Quart. J. Microscop. Sci. London, 61 : 217-274. 1916.
6. IMMS, A. D. Observations on the insect parasites of some Coccidae. II. On the Chalcid parasites of *Lecanium capreae*. Quart. J. Microscop. Sci. London, 63 : 293-374. 1918.
7. JAMES, H. C. The anatomy of a British phytophagous Chalcidoid of the genus *Harmolita* (*Isosoma*). Proc. Zool. Soc. London : 75-182. 1926.
8. LEONARD, M. D. A list of the insects of New York. Cornell Univ. Agr. Expt. Sta. Mem. 101. 1926.
9. MUESEBECK, C. F. W. *Monodontomerus aereus* Walker, both a primary and a secondary parasite of the brown-tail moth and the gypsy moth. J. Agr. Research, 43 : 445-460. 1931.
10. SNODGRASS, R. E. The thorax of Hymenoptera. Proc. U.S. Nat. Museum, 39 : 37-91. 1911.
11. SNODGRASS, R. E. Principles of insect morphology. McGraw-Hill Book Co., Inc., New York. 1935.

NOTE: Figs. 1-107 will be found on pp. 265-281.

EXPLANATION OF FIGURES

- FIG. 1. Dorsal view of the head. $\times 55$.
 FIG. 2. Lateral view of the head and mouth parts. $\times 55$.
 FIG. 3. Frontal view of the head. $\times 55$.
 FIG. 4. Frontal view of the head, specimen cleared and frons removed to show the tentorium. $\times 55$.
 FIG. 5. Posterior view of the head and mouth parts. $\times 55$.
 FIG. 6. Posterior view of the head, specimen cleared to show the tentorium. $\times 55$.

A, articulation; AN, antennifer; AS, antennal suture; ASO, antennal socket; AT, anterior tentorial pit; ATA, anterior tentorial arm; BS, basal segment; CD, cardo; CLP, clypeus; CT, corporotentorium; E, compound eye; ES, epistomal suture; FEX, frontal excavation; FOR, foramen magnum; FR, frons; GA, galea; GE, gena; GU, gula; ITR, internal ridge of the tentorium; LB, labrum; LC, lacinia; LIG, ligula; LO, lateral ocellus; LSC, ligular sclerite; MD, mandible; MO, median ocellus; MT, mentum; OC, occiput; OCC, occipital condyle; OCS, occipital suture; PDC, pedicel; PGE, postgena; POC, post-occiput; POCS, postoccipital suture; PRTL, parietal region; PT, posterior tentorial pit; PTA, posterior tentorial arm; RS, ring segment; SCP, scape; SGE, subgena; SGS, subgenal suture; SMT, submentum; SOS, subocular suture; ST, stipes; TL, tentorial lamella; VX, vertex.

- FIG. 7. Posterior view of the right maxilla. $\times 100$.
 FIG. 8. Medial view of the left maxilla. $\times 100$.
 FIG. 9. Lateral view of the right maxilla. $\times 100$.
 FIG. 10. Posterior view of the labium. $\times 100$.
 FIG. 11. Anterior view of the labium. $\times 100$.
 FIG. 12. Lateral view of the labium. $\times 100$.
 FIG. 13. Anterior view of the right mandible. $\times 100$.

FIG. 14. Posterior view of the right mandible. $\times 100$.

FIG. 15. Sixth segment of the flagellum of the female antenna. $\times 100$.

A, articulation; C, condyle; CD, cardo; GA, galea; HPH, hypopharynx; HSC, sclerite of the hypopharynx; LC, lacinia; LIG, ligula; LSC, ligular sclerite; MT, mentum; SP, sensory pit; ST, stipes.

FIG. 16. Lateral view of the prothorax. $\times 55$.

FIG. 17. Posterior view of the prothorax, showing the first spiracle. $\times 45$.

FIG. 18. Ventral view of the prothorax. $\times 45$.

FIG. 19. Dorsal view of the endoskeleton of the thorax. The specimen has been cleared and the nota removed. $\times 55$.

FIG. 20. Posterodorsal view of the prothorax of a cleared specimen. $\times 55$.

FIG. 21. Proepisternum and coxa, the former flattened in one plane. $\times 55$.

FIG. 22. Ventral view of the cleared prothorax with the head attached. $\times 55$.

A, articulation; AFU, arm of the furca; CV, cervical sclerite; CX, coxa; EPS, episternum; FA, arch of the furca; IPR, pleural ridge; IRP, internal ridge of the prepectus; MFU, manubrium of the furca; MTR, reduplication of the metasternum; OCC, occipital condyle; PHA, articulation of the pronotum with the head; PMA, articulation of the pronotum with the mesothorax; PRP, prepectus; PRT, protergum; PSR, reduplication of the prosternum; PWP, pleural wing process; SPI, first spiracle; STN', STN'', STN''', pro-, meso-, metasternum; ST1, first stigmatic trunk.

FIG. 23. Lateral view of the thorax. $\times 50$.

FIG. 24. Ventral view of the thorax showing the position of the coxae. $\times 50$.

FIG. 25. Dorsal view of the thorax. $\times 50$.

FIG. 26. Ventral view of the thorax. $\times 50$.

AAP, anterior alar plate; AB, postalar bridge; ANP, anterior notal wing process; CX, coxa; CXC, coxal cavity; D, dimple of the mesepimeron; EPM, epimeron; EPS, episternum; FP, furcal pit; MN, metanotum; MPA, articulation of the mesothorax with the prothorax; MSR, reduplication of the mesosternum; MTR, reduplication of the metasternum; PAP, posterior alar plate; PAR, parapsidal plate; PD, propodeum; PER, external rim of the parapsis; PF, parapsidal furrow; PNP, posterior notal wing process; PRP, prepectus; PRT, protergum; PS, pleural suture; PSCT, postscutum; PSS, suture of the postscutum; PWP, pleural wing process; SCL, scutellum; SCS, subcostal scale; SCT, scutum; SFL, postscutal flange; SP2, second spiracle; STN, sternum; TEG, tegula; TP, triangle of the prepectus; TR, trochanter; TSCS, transscutellar suture; TSS, transscutal suture; VS, scutoscuteular suture; 2-3S, abdominal sterna; 2-5T, abdominal terga; 2TR, second trochanter.

FIG. 27. Lateral view of the detached and cleared mesothorax. $\times 55$.

FIG. 28. Dorsal view of the detached and cleared mesothorax. $\times 55$.

FIG. 29. Ventral view of the cleared mesonotum. $\times 55$.

A, articulation; AAP, anterior alar plate; AB, postalar bridge; ACS, antecostal suture; ANP, anterior notal wing process; D, dimple of the mesepimeron; EP, internal spine of the epimeron; EPM, epimeron; EPS, episternum; IPR, pleural ridge; IWR, internal ridge supporting the wing process; MPA, articulation of the mesothorax with the prothorax; MSR, reduplication of the mesosternum; MSRA, process of the reduplication of the mesosternum; PAP, posterior alar plate; PAR, parapsidal plate; PER, external rim of parapsis; PF, parapsidal furrow; PFL, parapsidal flange; PN, mesopostnotum; PNP, posterior notal wing process; PR, internal parapsidal ridge; PRP, prepectus; PS, pleural suture; PSCT, postscutum; PSR, parapsidal submarginal ridge; PSS, suture of the postscutum; PWP, pleural wing process; SCL, scutellum; SCT, scutum; SFL, postscutal flange; SPS, pseudophragma of the postscutum; TEG, tegula; TSCS, transscutellar suture; TSS, transscutal suture; VR, internal ridge of the scutoscuteular suture; VS, scutoscuteular suture; X, articulating sclerite of the melanotum; IPH, prephragma; 2PH, postphragma.

FIG. 30. Posterodorsal view of the cleared mesopectus showing the endoskeleton. $\times 55$.

FIG. 31. Ventral view of the mesothorax. $\times 55$.

FIG. 32. Lateral view of the cleared mesopectus showing the endoskeleton. $\times 55$.

FIG. 33. Anteroventral view of the cleared mesonotum, showing the postnotum and its phragmata. $\times 55$.

FIG. 34. Ventroposterior view of the metathorax and propodeum. $\times 55$.

A, articulation; AB, postalar bridge; AFU, furcal arm; D, dimple of the mesepimeron; CX, coxa; CXC, coxal cavity; EP, internal spine of the epimeron; EPM, epimeron; EPS, episternum; FA, furcal arch; FP, furcal pit; IPR, pleural ridge; IRP, internal ridges of the prepectus; IWR, internal ridge of the wing process; MFU, furcal manubrium; MPA, articulation of the mesothorax with the prothorax; MSR, reduplication of the mesosternum; MSRA, process of the reduplication of the mesosternum; MTR, reduplication of the metasternum; PAP, posterior alar plate; PAR, parapsidal plate; PD, propodeum; PN, mesopostnotum; PNP, posterior notal wing process; PPS, pseudophragma of the postnotum; PRP, prepectus; PS, pleural suture; PSCT, postscutum; PWP, pleural wing process; SCL, scutellum; SPS, pseudophragma of the postscutum; STN, sternum; TEG, tegula; TP, triangle of prepectus; TSS, transscutal suture; VR, internal ridge of the scutoscuteellar suture; 1PH, prephragma; 2PH, postphragma; 2S, 3S, abdominal sterna; 3T, abdominal tergum.

FIG. 35. Dorsal view of the petiole. $\times 55$.

FIG. 36. Lateral view of the petiole. $\times 55$.

FIG. 37. Anterior view of the petiole. $\times 55$.

FIG. 38. Dorsal view of the metathorax and propodeum. $\times 55$.

FIG. 39. Anteroventral view of the cleared metathorax and propodeum. $\times 55$.

FIG. 40. Posterior view of the cleared metathorax and propodeum. $\times 55$.

FIG. 41. Anterior view of the cleared metathorax and propodeum. $\times 55$.

A, articulation; AFU, furcal arm; ANP, anterior notal wing process; CX, coxa; CXC, coxal cavity; EPM, epimeron; EPS, episternum; FOP, foramen of the propodeum; INP, inflection of the metapectus; IPR, pleural ridge; MFU, furcal manubrium; MN, metanotum; MTR, reduplication of the metasternum; PD, propodeum; PNP, posterior notal wing process; PS, pleural suture; PWP, pleural wing process; SP2, second spiracle; STN, sternum; 3S, abdominal sternum.

FIG. 42. Dorsal view of the pretarsus. $\times 250$.

FIG. 43. Ventral view of the pretarsus. $\times 250$.

FIG. 44. Lateral view of the pretarsus. $\times 250$.

FIG. 45. Antenna of the male. $\times 55$.

FIG. 46. Antenna of the female. $\times 55$.

FIG. 47. Reticulation and setae of the scutum. $\times 250$.

FIG. 48. Anterior view of the right prothoracic leg. $\times 35$.

FIG. 49. Anterior view of the right mesothoracic leg. $\times 35$.

FIG. 50. Anterior view of the right metathoracic leg. $\times 35$.

AR, arolium; BS, basal segment; CA, camera; CC, calcar; CX, coxa; FM, femur; OR, orbicula; PDC, pedicel; PTAR, pretarsus; RS, ring segment; SCP, scape; TAR, tarsus; TB, tibia; TR, trochanter; UN, unguis; UNG, unguitactor; 2TR, second trochanter.

FIG. 51. Lateral view of the coxopleural articulation of the prothoracic leg. $\times 100$.

FIG. 52. Lateral view of the coxopleural articulation of the mesothoracic leg. $\times 100$.

FIG. 53. Lateral view of the coxopleural articulation of the metathoracic leg. $\times 100$.

FIG. 54. Dorsolateral view of the coxotrochanteral and trochanterofemoral articulations of the prothoracic leg. $\times 100$.

FIG. 55. Ventral view of the coxotrochanteral and trochanterofemoral articulations of the mesothoracic leg. $\times 100$.

FIG. 56. Ventral view of the coxotrochanteral and trochanterofemoral articulations of the metathoracic leg. $\times 100$.

FIG. 57. Dorsal view of the tibiofemoral articulation of the prothoracic leg. $\times 100$.

FIG. 58. Dorsal view of the tibiofemoral articulation of the mesothoracic leg. $\times 100$.

FIG. 59. Dorsal view of the tibiofemoral articulation of the metathoracic leg. $\times 100$.

FIG. 60. Lateral view of the tibiotalarsal articulation of the prothoracic leg. $\times 100$.

FIG. 61. Lateral view of the tibiotarsal articulation of the mesothoracic leg. $\times 100$.

FIG. 62. Tibiotarsal joint showing the condyle. $\times 100$.

FIG. 63. Lateral view of the tibiotarsal articulation of the metathoracic leg. $\times 100$.

A, articulation; C, condyle; CC, calcar; CX, coxa; EPS, episternum; FM, femur; PD, propodeum; TAR, tarsus; TB, tibia; TR, trochanter; 2TR, second trochanter.

FIG. 64. Frenulum. $\times 250$.

FIG. 65. Mesothoracic wing. $\times 40$.

FIG. 66. Metathoracic wing. $\times 40$.

FIG. 67. Dorsal view of the axillary sclerites of the mesothoracic wing. $\times 90$.

FIG. 68. Dorsal view of the axillary sclerites of the metathoracic wing. $\times 90$.

FIG. 69. Lateral view of the thorax with the wings elevated to show the articulations with the pleura. $\times 80$.

ANP, anterior notal wing process; AX1, AX2, AX3, axillaries; BAL, basalar; CU', first cubital branch; EPM, epimeron; EPS, episternum; FL, frenulum; M, median vein; M', basal sclerite of the median vein; MV, marginal vein; PD, propodeum; PNP, posterior notal wing process; PMV, postmarginal vein; PSCT, postscutum; PWP, pleural wing process; R, radial vein; R', first radial branch; SC, subcosta; SCL, scutellum; SCS, subcostal scale; SMV, submarginal vein; SP2, second spiracle; STV, stigmal vein; TEG, tegula; WR, sclerotized rib of the mesothoracic wing; X, articulating sclerite of the metanotum.

FIG. 70. Lateral view of the female abdomen with the sternites depressed and the sting revealed. $\times 30$.

FIG. 71. Lateral view of the male abdomen with the aedeagus exerted. $\times 30$.

FIG. 72. Dorsal view of the female abdomen. $\times 30$.

FIG. 73. Ventral view of the female abdomen. $\times 30$.

FIG. 74. Dorsal view of the male abdomen with the aedeagus exerted. $\times 30$.

FIG. 75. Ventral view of the male abdomen with the aedeagus exerted. $\times 30$.

AED, aedeagus; AES, aedeagus sheath; ALP, anal papilla; CX, coxa; CXC, coxal cavity; EPS, episternum; MTR, reduplication of the metasternum; PAL, sensory palp; PD, propodeum; SPL, sensory plate; STV, stylet; STYS, stylet sheath; 2-9S, abdominal sterna; 2-10T, abdominal terga.

FIG. 76. Dorsal view of the cleared ovipositor. $\times 55$.

FIG. 77. Ventral view of the proximal end of the cleared ovipositor with the plates spread. $\times 55$.

FIG. 78. Inside view of the right half of the cleared ovipositor with the parts spread. $\times 55$.

FIG. 79. Lateral view of the terminal portion of the stylet sheaths showing the teeth. $\times 250$.

FIG. 80. Transverse section of the stylets and stylet sheaths. $\times 500$.

FIG. 81. The sclerite of the vagina. $\times 55$.

FIG. 82. Lateral view of the cleared ovipositor. $\times 55$.

A, articulation; ALP, anal papilla; ET, egg-tube; FP, fulcral plate; IP, inner plate; IPB, bridge of the inner plate; IPP, pivoting sclerite of the inner plate; IPR, rib of the inner plate; OP, outer plate; OPR, rib of the outer plate; PAL, sensory palp; RP, rotatory process; RPR, transverse ribs of the rotatory processes; SPL, sensory plate; STV, stylet; STYS, stylet sheath; 9-10T, abdominal terga.

FIG. 83. The female reproductive system from a dorsal aspect, the ovaries being displaced outwards and backwards. $\times 55$.

FIG. 84. Dorsal view of the poison apparatus. $\times 55$.

FIG. 85. Dorsal view of the male reproductive system. $\times 55$.

FIG. 86. Dorsal view of the aedeagus, cleared and removed from the sheath. $\times 55$.

FIG. 87. Dorsal view of the aedeagus and its sheath. $\times 55$.

FIG. 88. Ventral view of the aedeagus and its sheath. $\times 55$.

ACG, acid gland; ACR, reservoir of acid gland; AED, aedeagus; AEDA, aedeagus arm; AES, aedeagus sheath; AGS, accessory glands of spermatheca; ALG, alkaline gland; CH,

chorion; CL, clasper; COV, common oviduct; DE, ductus ejaculatorius; EG, egg; FE, follicular epithelium; FP, fulcral plate; GER, germarium; GM, glandula mucosa; GP, genital pore; IP, inner plate; NC, nurse cells; OP, outer plate; OV, oviduct; OVL, ovariole; SPL, sensory plate; SPM, spermatheca; STY, stylet; STYS, stylet sheath; TT, testis; VAG, vagina; VD, vas deferens; VI, valva interna; VIT, vitellarium; VSC, sclerite of the vagina; VSM, vesicula seminalis; IAG, primary accessory gland; 2AG, secondary accessory gland; 9T, 10T, abdominal terga.

FIG. 89. Section of the salivary gland of the hypopharynx. $\times 500$.

FIG. 90. Sagittal section of the duct of the thoracic salivary gland. $\times 500$.

FIG. 91. Transverse section of the crop. $\times 500$.

FIG. 92. Transverse section of the proventriculus. $\times 500$.

FIG. 93. Dorsal view of the alimentary canal. $\times 55$.

FIG. 94. Lateral view of the pharynx showing the pharyngeal plate. $\times 55$.

FIG. 95. Sagittal section of the inner wall of the pharynx. $\times 500$.

FIG. 96. Coronal section of the ventriculus. $\times 500$.

FIG. 97. Transverse section of a Malpighian tubule. $\times 500$.

FIG. 98. Transverse section of the intestine. $\times 500$.

ALP, anal papilla; BM, basement membrane; CM, circular muscles; CR, crop; CX, coxa; EPH, epipharynx; ETH, epithelium; FAT, fat; IN, intima; INT, intestine; LB, labrum; LD, duct of the labial salivary gland; LGL, labial salivary gland; LM, longitudinal muscles; MAL, Malpighian tubules; OE, oesophagus; OP, outer plate; PHP, pharyngeal plate; PHY, pharynx; PVT, proventriculus; RC, rectum; RGL, rectal gland; SPL, sensory plate; VT, ventriculus; 3-8S, abdominal sterna; 9-10T, abdominal terga.

FIG. 99. Dorsal view of the tracheal system of the female. $\times 55$.

CAT, anterior connective; CDO, dorsal connective; CMD, middle connective; CPT, posterior connective; CX, coxa; SPI, 2, 3, spiracles; STY, stylets; ST1, 2, 3, stigmatic branches; TLA, longitudinal abdominal trunk; 3-8S, abdominal sterna; 2T, 8T, abdominal terga.

FIG. 100. Dorsal view of the nervous system of the female. $\times 55$.

FIG. 101. Frontal aspect of the brain. $\times 55$.

FIG. 102. Dorsal view of the tracheal and nervous systems in the male abdomen. $\times 55$.

AES, aedeagus sheath; CPT, posterior connective; CX, coxa; GAC, accessory ganglion; GA1, GA2, abdominal ganglia; GDO, supraoesophageal ganglion; GT1, 2, 3, thoracic ganglia; GVO, suboesophageal ganglion; HGL, sublingual salivary gland; LD, duct of the labial salivary glands; NA, antennary nerve; NAT, nerve to the terminal floor of the abdomen; ND, nerve to the dorsolongitudinal muscles; NF, frontal commissure; NL1, 2, 3, leg nerves; NM, nerve to the direct wing muscles; NMD, mandibular nerve; NOC, ocellar nerve; NR, nerve to the rectum; NW1, 2, nerves to the wings; OP, outer plate; OPL, optic lobe; SP3, spiracle; STY, stylet; ST3, stigmatic trunk; TLA, longitudinal abdominal trunk; 3-8S, abdominal sterna; 2T, 8T, abdominal terga.

FIG. 103. Transverse section of the brain. $\times 90$.

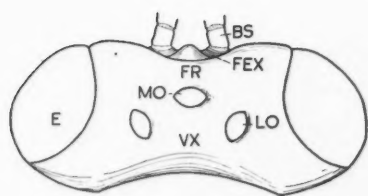
FIG. 104. Coronal section of the brain. $\times 90$.

FIG. 105. Sagittal section of the brain. $\times 90$.

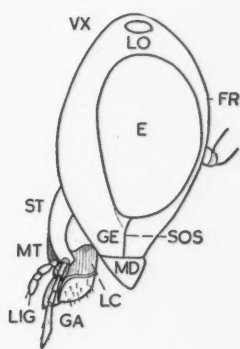
FIG. 106. Sagittal section of the brain showing the pharynx and oesophagus. $\times 90$.

FIG. 107. Sagittal section of the brain. $\times 90$.

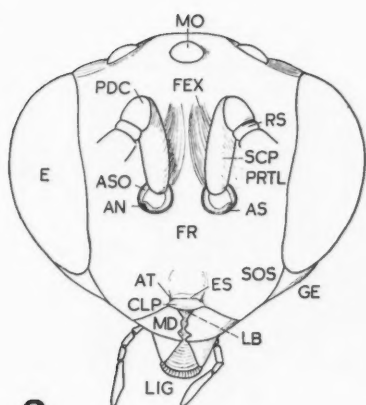
CB, central body; COM, constrictor muscles; CP, mushroom body; CYX, calyx; DM, dilator muscles; EOPT, epipticon; EPH, epipharynx; GDO, supraoesophageal ganglion; GF, frontal ganglion; GVO, suboesophageal ganglion; HGL, sublingual gland; HPH, hypopharynx; LB, labrum; LD, duct of labial salivary glands; LIG, ligula; MO, median ocellus; NF, frontal commissure; NL, labial nerve; NMD, mandibular nerve; NMX, maxillary nerve; NOC, ocellar nerve; NRE, recurrent nerve; NV, ventral nerve cord; OCC, occipital condyle; OE, oesophagus; OPT, opticon; PHY, pharynx; POPT, peripticon; POR, preoral cavity; 1BR, protocerebrum; 2BR, deutocerebrum; 3BR, tritocerebrum.



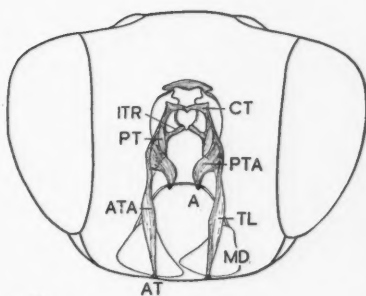
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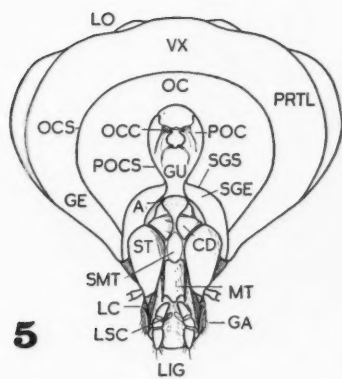
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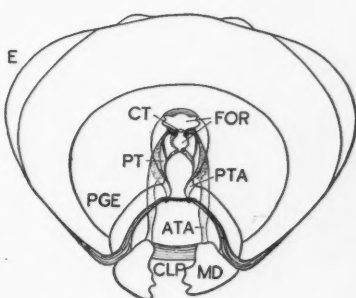
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4

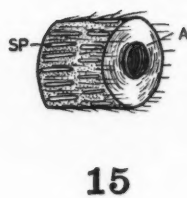
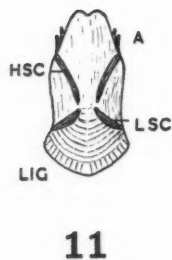
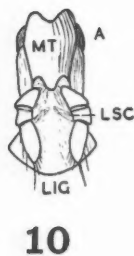
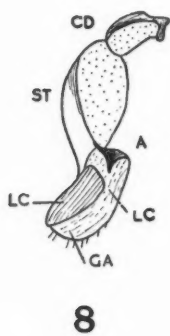
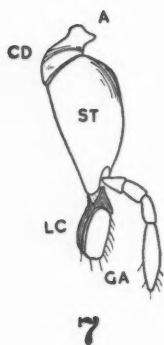


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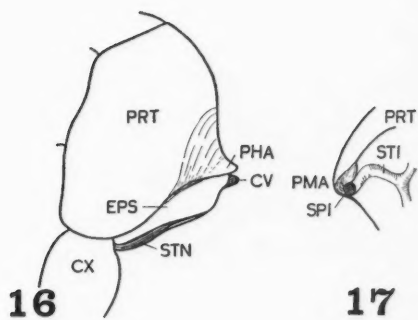


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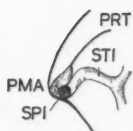
FIGS. 1 TO 6.



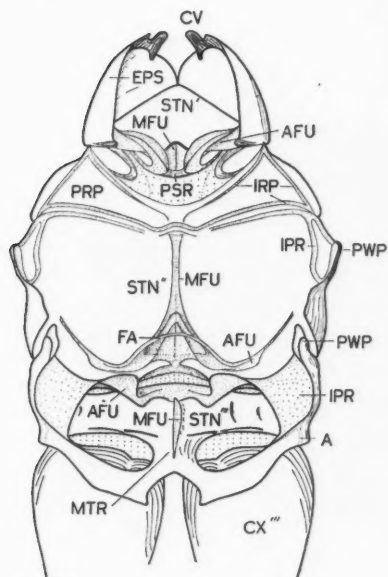
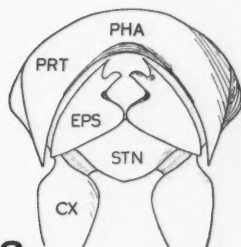
FIGS. 7 TO 15.



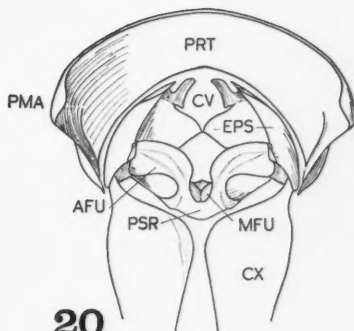
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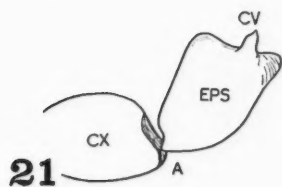
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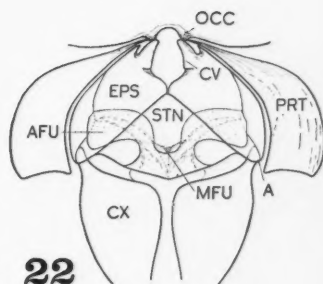
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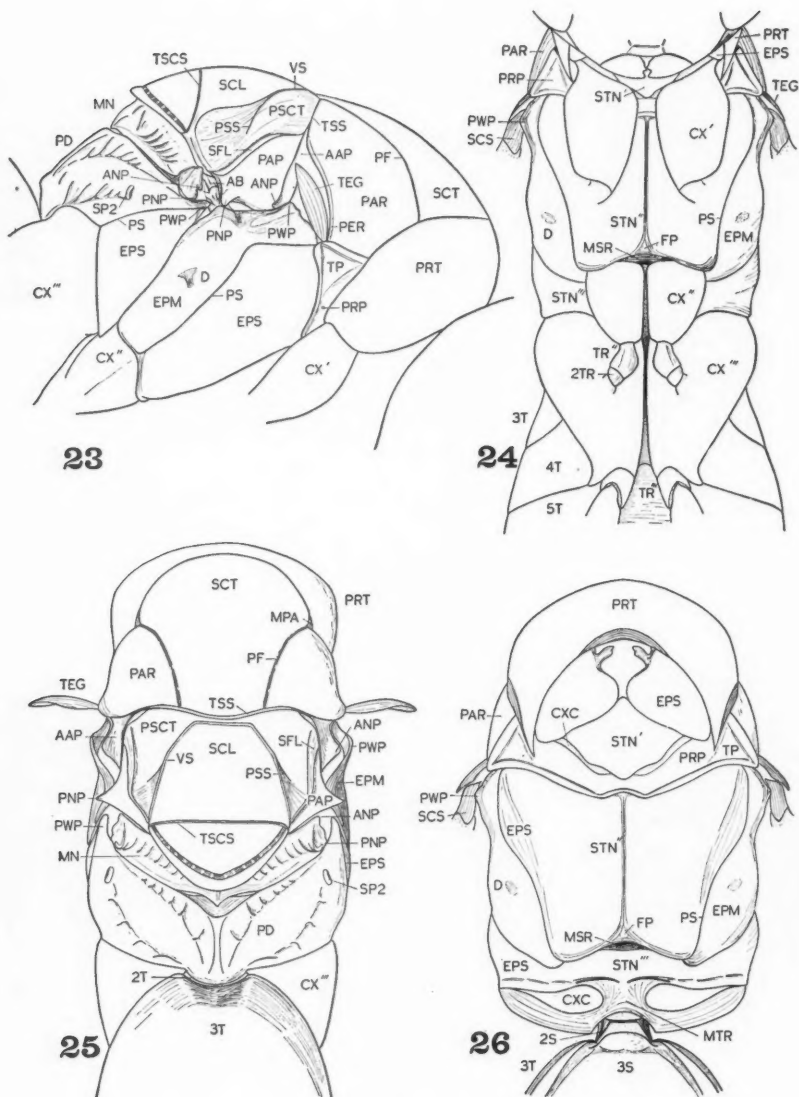


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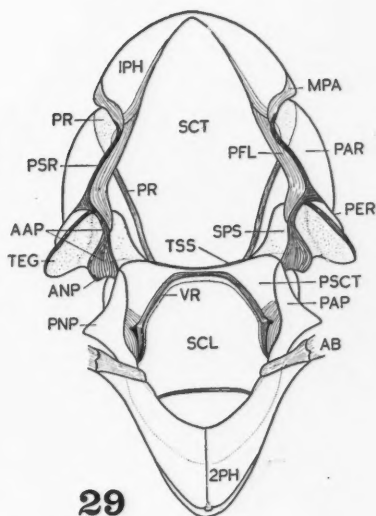
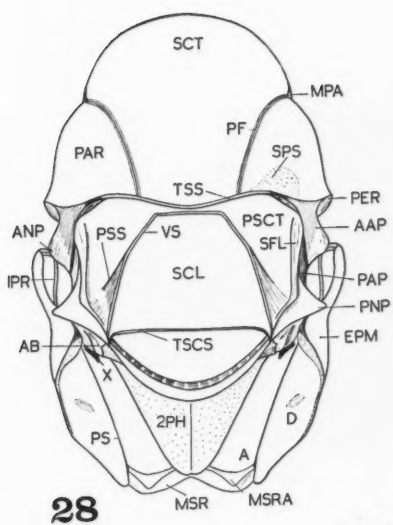
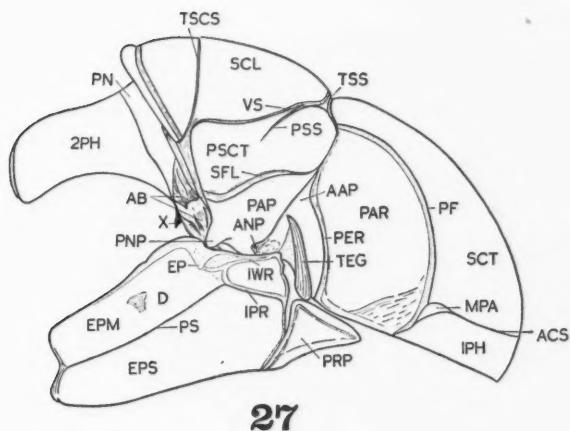


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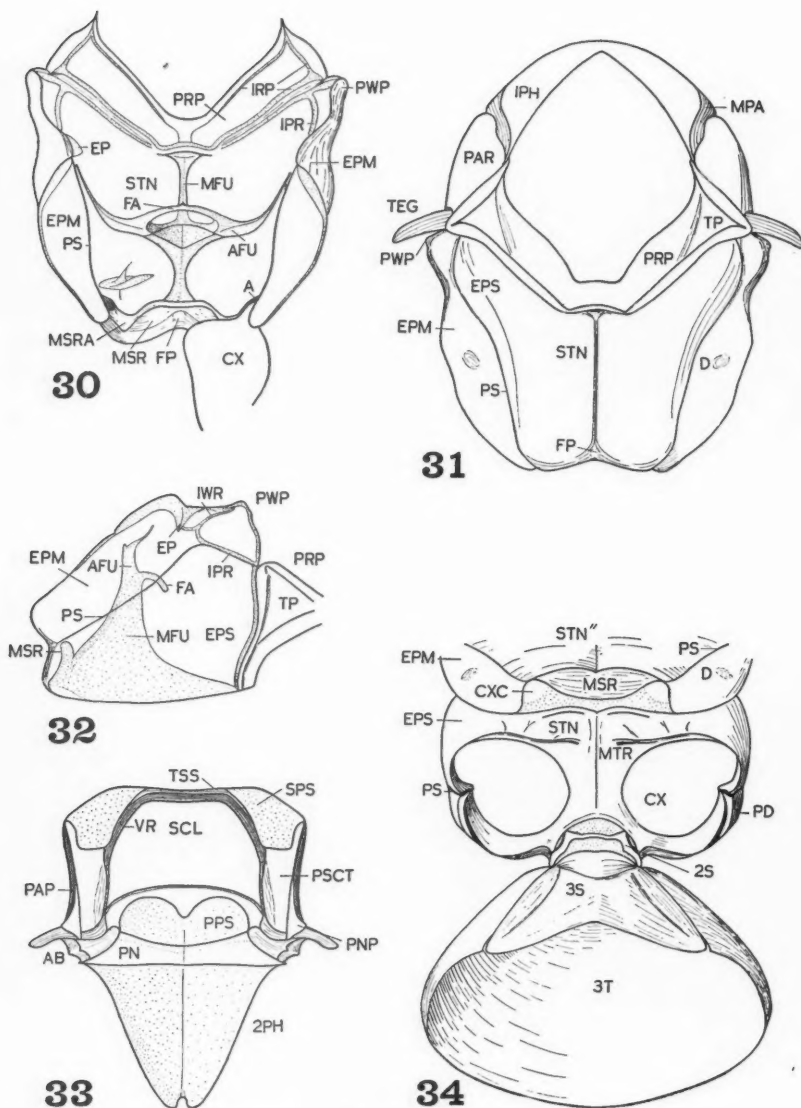
FIGS. 16 TO 22.



FIGS. 23 TO 26.



FIGS. 27 TO 29.



FIGS. 30 TO 34.



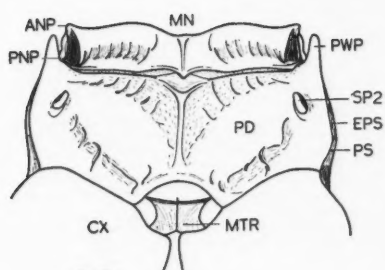
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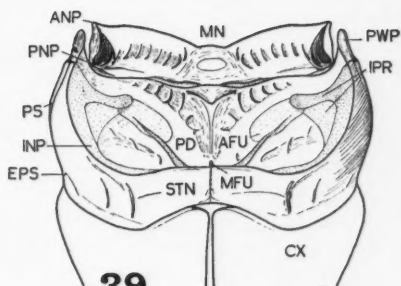
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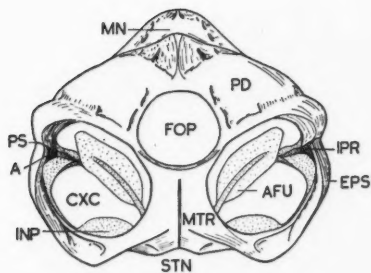
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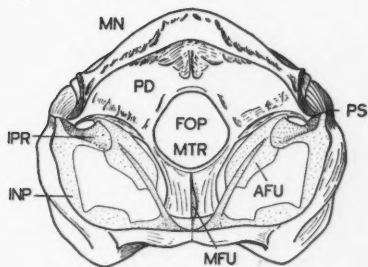
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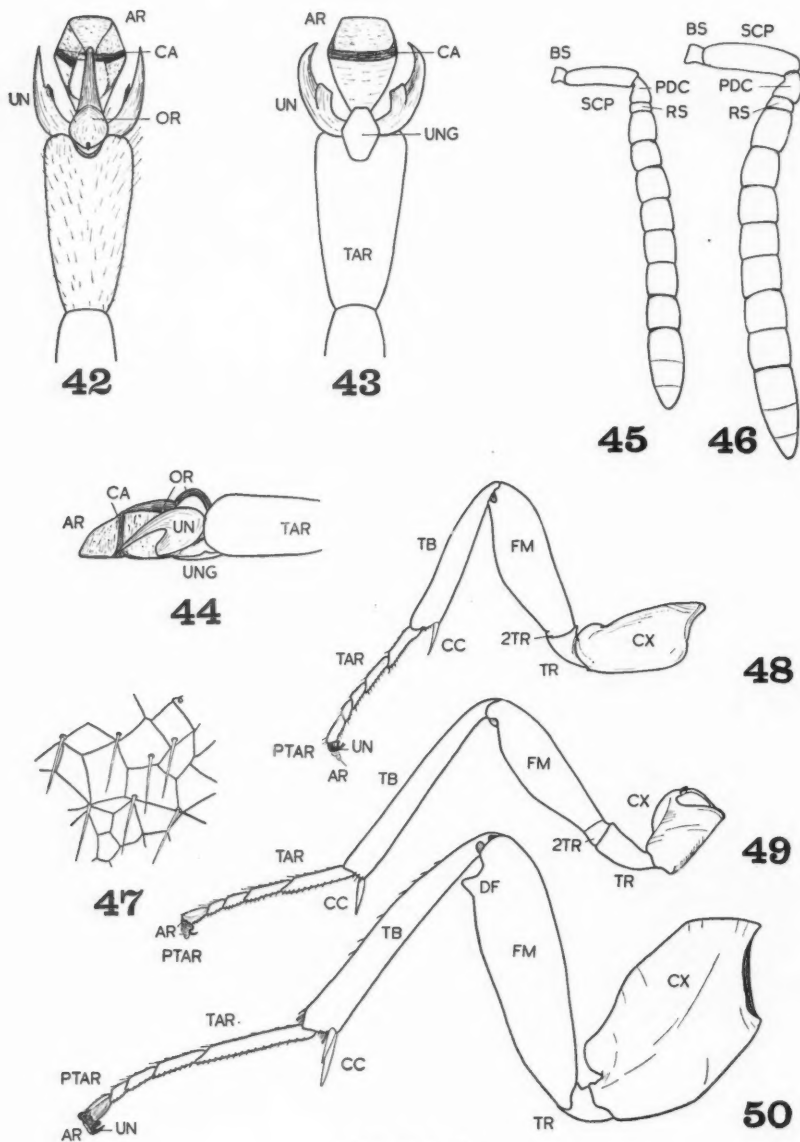


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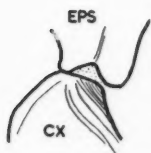


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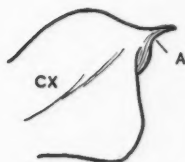
FIGS. 35 TO 41.



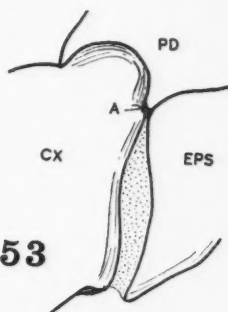
FIGS. 42 TO 50.



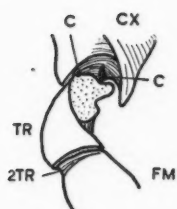
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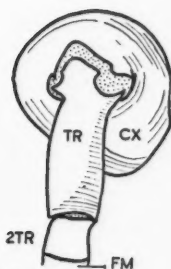
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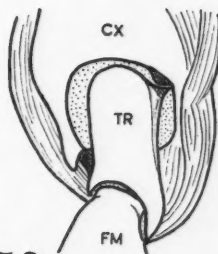
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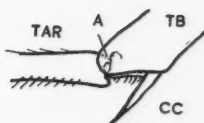
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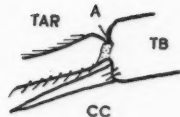
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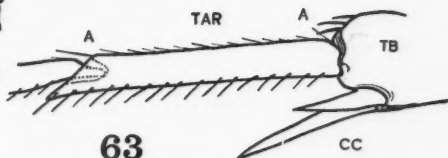
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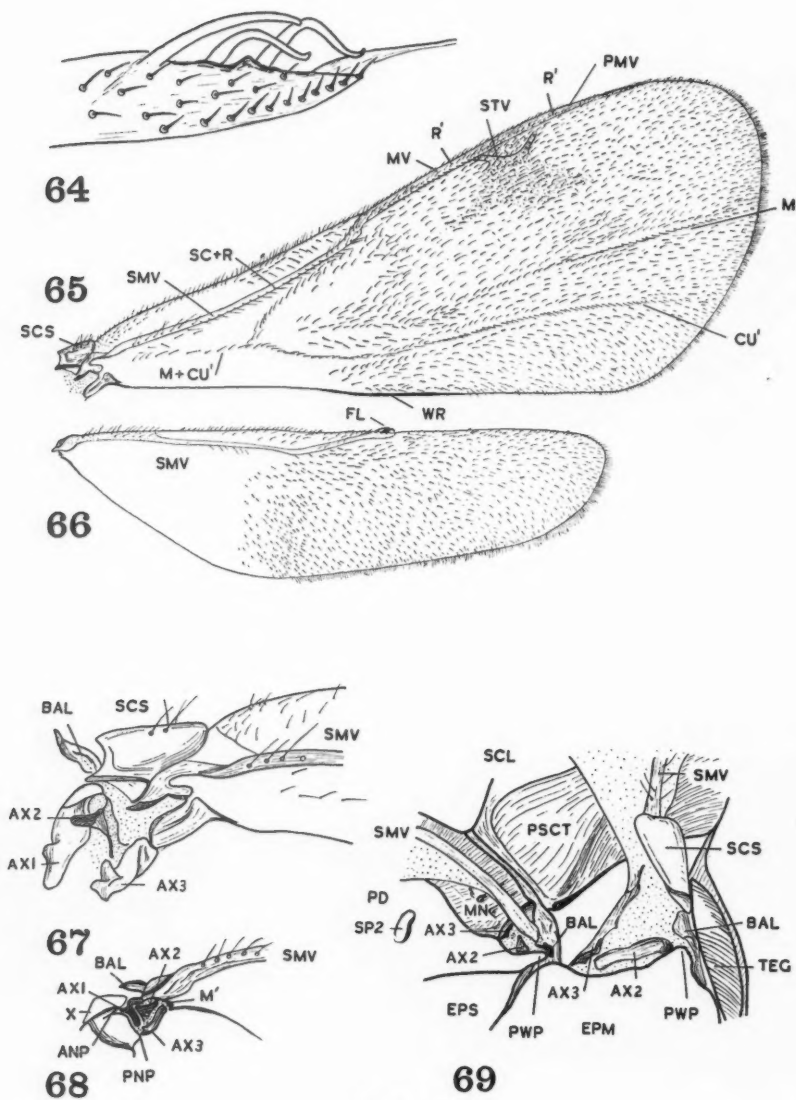


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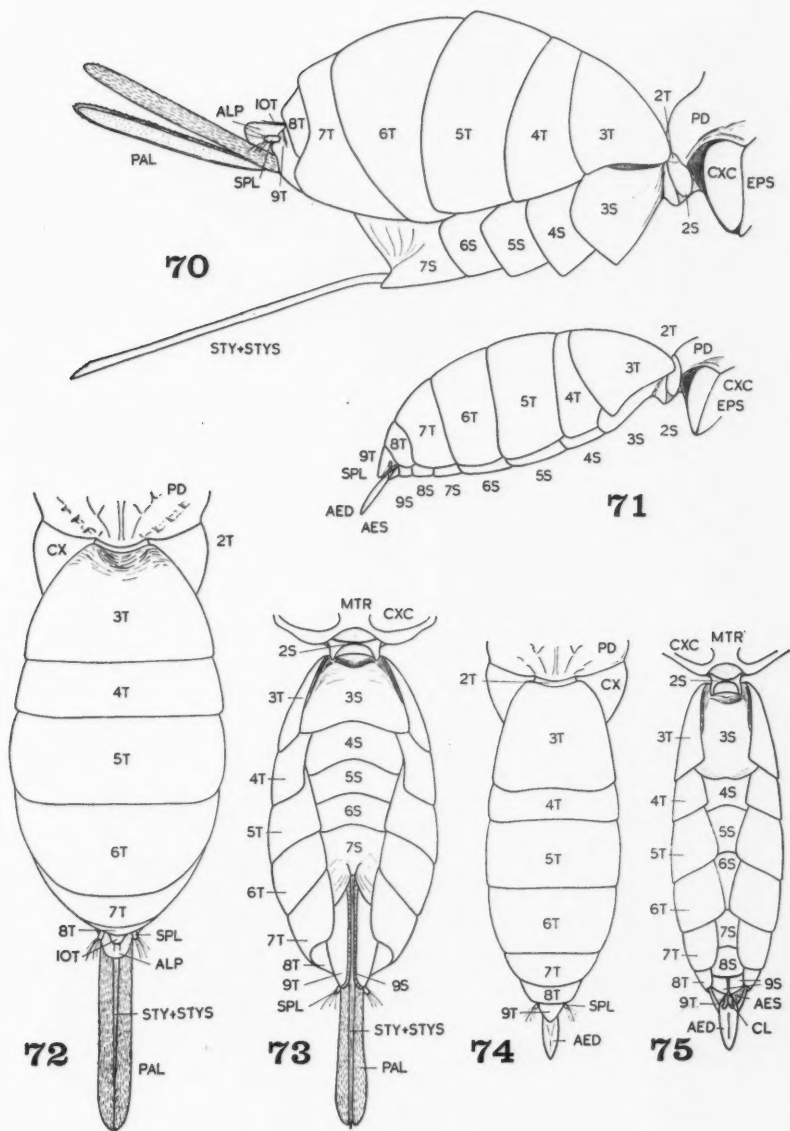


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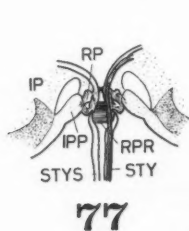
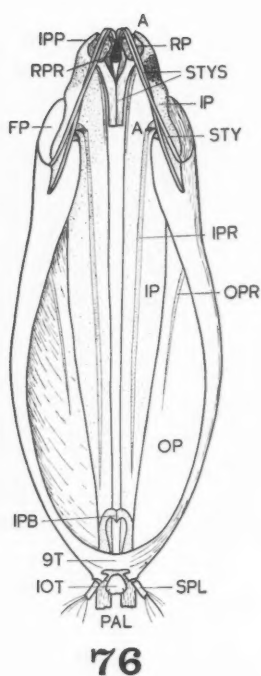
FIGS. 51 TO 63.



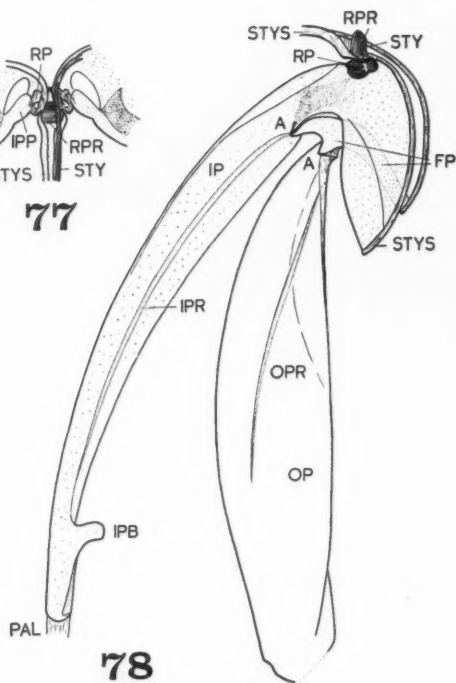
FIGS. 64 TO 69.



FIGS. 70 TO 75.



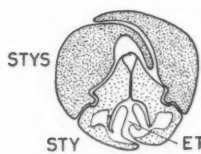
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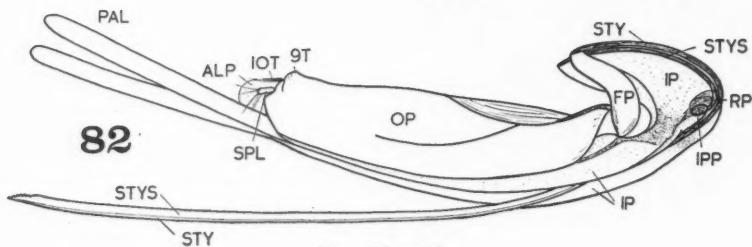
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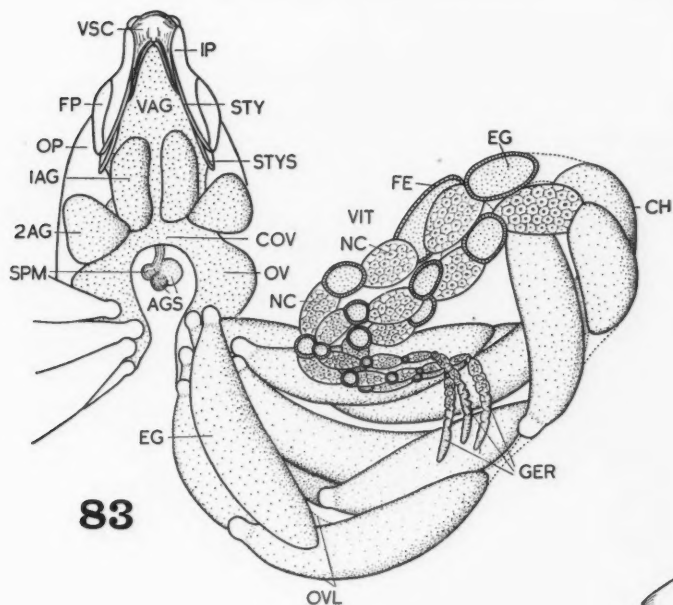


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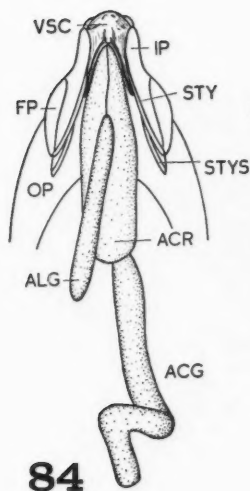


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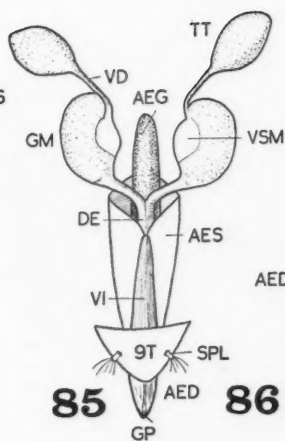
FIGS. 76 TO 82.



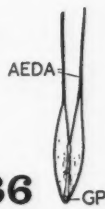
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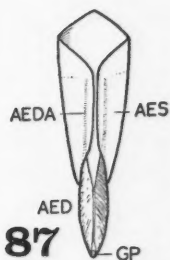
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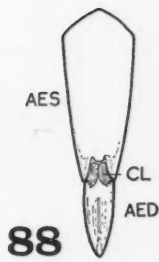
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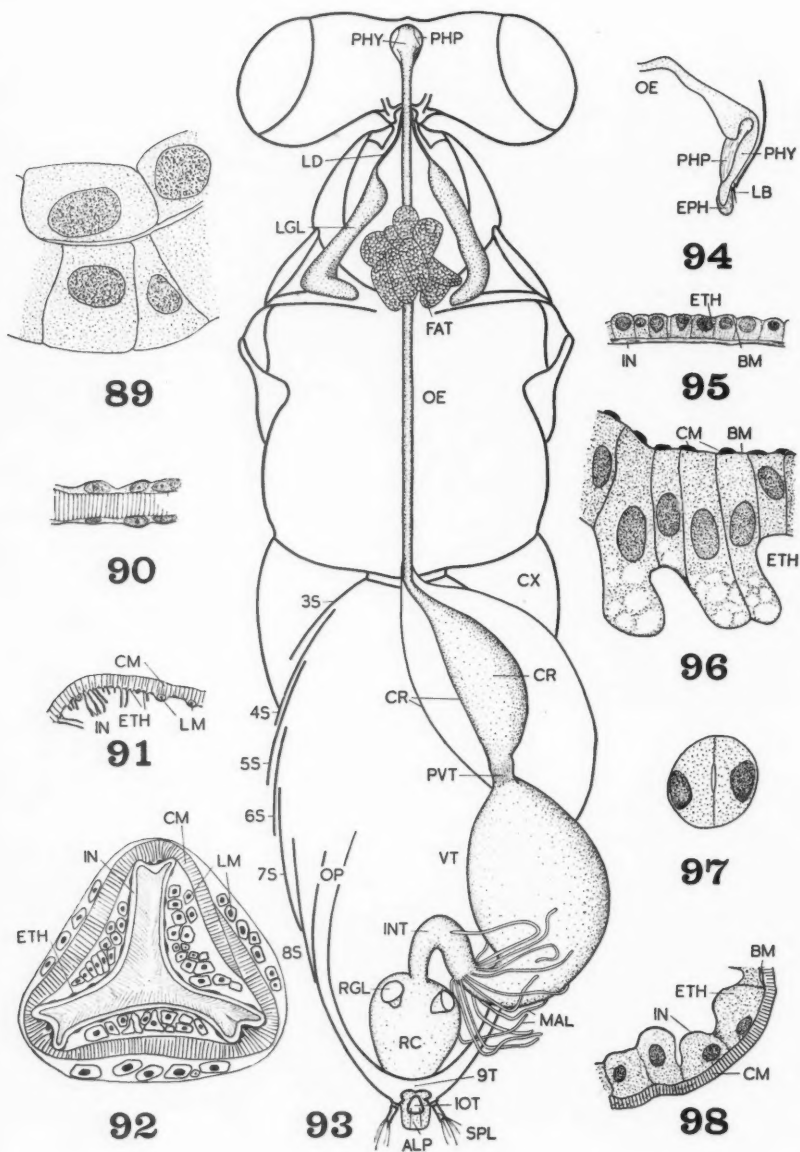


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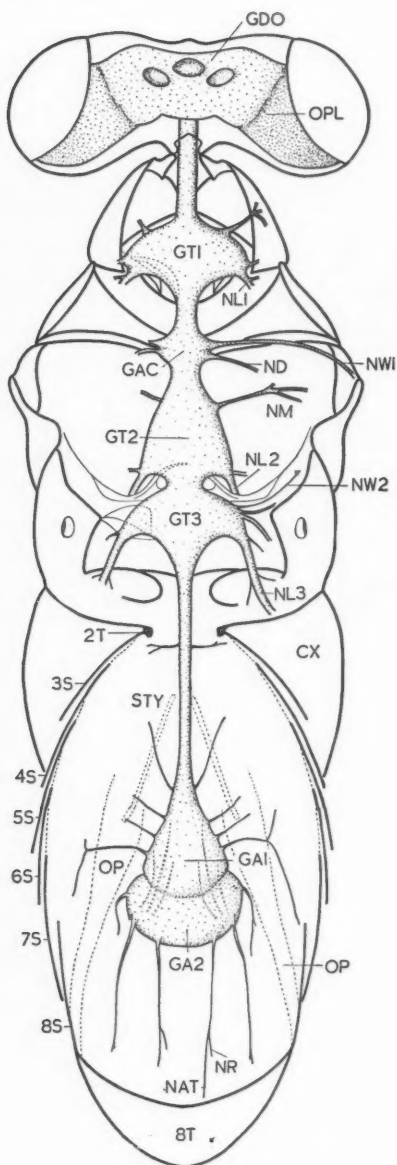


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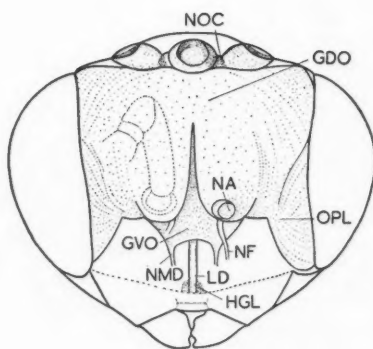
FIGS. 83 TO 88.



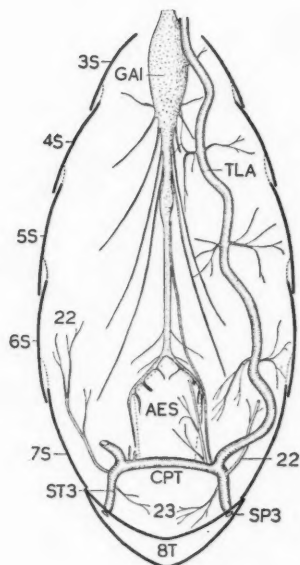
FIGS. 89 TO 98.



100

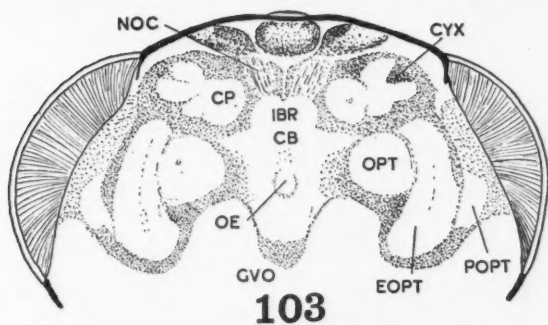


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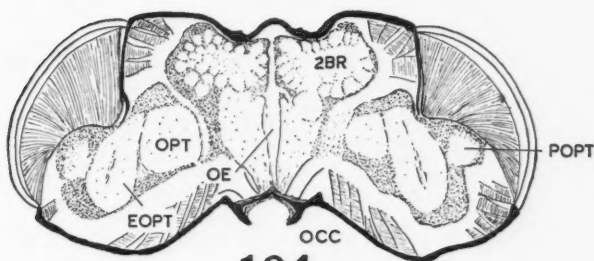


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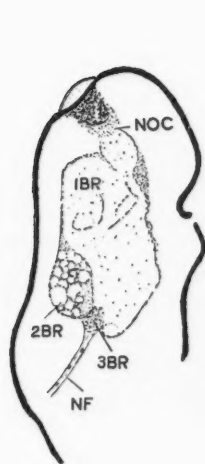
FIGS. 100 TO 102.



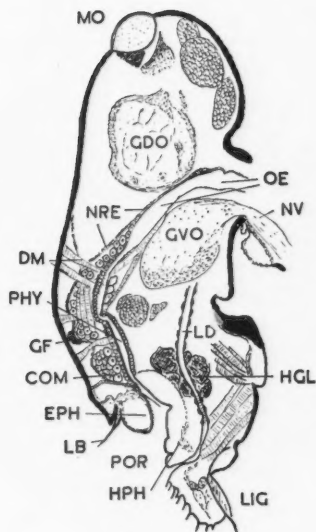
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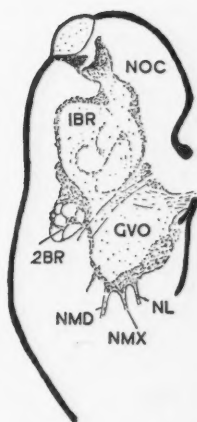
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105



106



107

FIGS. 103 TO 107.

THE BIOLOGICAL ACTIVITY OF DDT AND RELATED COMPOUNDS¹BY H. C. BROWNING,² F. C. FRASER,³ S. K. SHAPIRO,⁴ I. GLICKMAN,⁴ AND
M. DUBRÔLE⁵

Abstract

Sixty-eight compounds were examined for insecticidal activity, using *Drosophila melanogaster* as the test insect and the inner sides of glass containers as the test surface. The compounds included series of halogen analogues, reduced compounds, carbinols and their esters, ethanones, diphenylamines, and the gamma isomer of cyclohexane hexachloride. Activity was shown especially by the fluorine analogues and carbinol esters. Some compounds were briefly examined for mammalian toxicity.

The findings of other workers on the more important compounds are discussed and compared with those presently reported. No constant relation could be established between insecticidal activity and mammalian toxicity, or ease of dehydrochlorination. Low oil solubility was associated with low insecticidal potency (four compounds). No clear association of fat solubilizing or toxic properties with particular parts of the molecule was found.

The essentials of an active molecular structure are a two-carbon chain with one para-substituted phenyl ring on carbon 1 and a di- or tri-halogen group on a saturated carbon 2. Steric factors are also important.

Introduction

Subsequent to the discovery of the insecticidal properties of DDT, a number of investigations have been made of its isomers, analogues, and more distantly related compounds (3, 4, 5, 6, 7, 8, 10, 11, 19, 20, 22, 23, 27). Part of this work has been directed towards the finding of other insecticides, possibly with special properties differing from those of DDT, and part towards the elucidation of the relationship between chemical structure and potency.

From 1944 to 1946 a series of such compounds was synthesized in the Department of Chemistry, McGill University by Drs. D. L. Garmaise, G. T. Barry, and H. L. White (1, 25, 31) under the direction of Dr. R. Boyer. The present account deals with the testing of these substances together with some fluorine analogues (13) and diphenylamines supplied by Drs. S. Kirkwood and J. R. Dacey and a few compounds from industrial sources. This work was primarily a screening procedure, adapted for the rapid determination of insecticidal potentialities. Proverbs and Morrison (23) have given an account of more detailed tests of some of the same compounds using similar methods.

Methods

The test surface was the inside of a glass container on which a thin film of compound was left by evaporation from acetone. For each test a standard

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Contribution from the Department of Genetics, McGill University, Montreal, Que., with financial assistance from the National Research Council of Canada.

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³ Lecturer, Department of Genetics, McGill University.

⁴ Graduate Assistant, Department of Genetics, McGill University.

⁵ Undergraduate Assistant, Department of Genetics, McGill University.

solution was made containing 1 mgm. of pure DDT* per 100 cc. of acetone. The inner surfaces of half-pint milk bottles or of shell vials (7 by 2 cm.) were wet with the solution and dried at room temperature for approximately 20 hr. From 5 to 15 bottles or 50 to 100 vials were prepared. An identical series of test containers were made for each concentration of the compound under consideration. Usually three concentrations were tested initially: 2.5, 25, and 100 mgm. per 100 cc.

The test insects were adult *Drosophila melanogaster* four to six days after emergence. When vials were used as test containers 15 flies were placed in each of 50 to 100 vials; when bottles were used 100 flies were placed in each of 5 to 10 bottles. The two methods yielded results that did not differ significantly. The containers were stoppered with cotton wool plugs moistened with 5% aqueous molasses. They were incubated at 25° C. for 18 hr. when mortality counts were made. Each mortality percentage therefore refers to from 500 to 1500 flies scattered in from 5 to 100 separate groups. Control mortalities from containers treated with acetone alone were 2% or less.

In this way mortalities obtained using solutions of known concentrations of a given compound were compared with that obtained using a standard solution of pure DDT. The relative potency of a given compound was defined as the reciprocal of that concentration of the compound that produced approximately the same mortality as a 1 mgm. % solution of DDT under the conditions of the test. Compounds were often retested at different concentrations to obtain a mortality figure closer to that of the standard than was obtained in the initial test. They were also often retested as a check on the first results.

The test method (2) was a modification of that described by Morrison (18).

Results

The compounds that were tested are listed below. They are arranged according to structure rather than insecticidal potency. Those compounds that are discussed in later sections have been given a synonym. Insecticidal potencies relative to DDT have been inserted from Table I.

Table I lists the percentage mortalities for each test of each compound together with the figure for the corresponding standard. Compounds with an activity of 2/5ths to 1/10th of DDT were arbitrarily regarded as "good"; of 1/50th to 1/100th as "fair"; and of 1/250th to 1/500th as "poor". Compounds giving no mortality at a concentration of 500 mgm. % were considered to be totally inactive. At this concentration the film of compound on the glass of the test container is grossly visible and is considered equivalent to an unlimited supply of the substance.

* M.p. 108 to 109° C.; thrice recrystallized from ethanol and 100% pure by chemophysical assay.

TABLE I

PERCENTAGE MORTALITIES AMONG *Drosophila melanogaster* ON EXPOSURE TO DDT AND RELATED COMPOUNDS

Compound	No. of flies per test	Percentage mortalities with concentration in mgm. %										Relative potency	
		DDT	Compounds										
		1	2.5	5	10	25	50	75	100	250	500		
I. DDT	1500 750 500 500	14 12 34 39	53 54 71 79			61 72 79 82			77 89 92 99			1	
III. Fluoro	750 600	14 18	13 9		52		100		100			2/5 to 1/5	
IV. Bromo	500	41					7		19	24	40	1/500	
VI. Trifluoro	750 500	14 52	—			—		15	21 52			1/100	
VII. Tribromo	500 500	34 52	—			—			4		23	<1/500	
VIII. Fluorotrifluoro	750 600	14 18	—			—			89			1/75 to 1/100	
XI. Methyl	500	41	6	17	25	29			45			1/100	
XV. Monochlor	1500 1000 500	24* 41 52	4	4	12 17	25	27			44	48	1/50 to 1/100	
XIX.	500	38		4					46		92	>1/100	
XX.	500	39					36		98		100	1/50	
XXI. Carbinol	750 500 500 500 1000	49* 39 34 39 36	—	—	4	—	26 12 35 33		80 91 98		100 100	1/50	
XXII. l-Carbinol	1000 500	33 36					25 72 71					1/25 to 1/50	
XIII. d-Carbinol	1000 500	33 36					22 34			83		<1/50	
XXIV. Fluor-carbinol	750 600	14 18	12 10		58		100		100			2/5 to 1/5	
XXVI. Methoxy-carbinol	500 500	39 23					18 25			100	100	1/50	
XXVII. Ethoxy-carbinol	500 500	38 74		6		7	16	27	82		99	1/50 to 1/75	
XXVIII. Acetoxy-carbinol	500 1000 500	34 39 52	9 6	33			53			76		1/5 to 1/10	

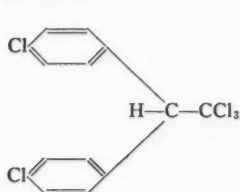
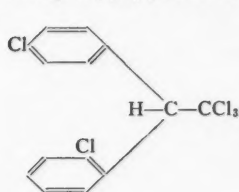
* 2.5 mgm. %.

TABLE I—*Concluded*PERCENTAGE MORTALITIES AMONG *Drosophila melanogaster* ON EXPOSURE TO DDT AND RELATED COMPOUNDS—*Concluded*

Compound	No. of flies per test	Percentage mortalities with concentration in mgm. %										Relative potency	
		DDT	Compounds										
			1	2.5	5	10	25	50	75	100	250		500
XXXII. Butoxy-carbinol	500	24		—		—			16		29	1/100 to 1/500	
XXXIX.	1000	45	—			—			—		99	1/250	
	500	51							17	53	98		
XL.	1000	45	—			—			—		99	1/250 to 1/500	
	500	51								24	99		
XLI.	500	24					45			100	100	>1/50	
XLII.	500	24					44			99	100	>1/50	
L.	500	40									35	<1/500	
	500	57								21	28		
LI.	500	40									50	1/500	
	500	57								16	25		
LVII.	500	38	—								44	1/500	
LXIV.	500	24								13	18	<1/500	
LXVII.	500	24								35	32	>1/250	

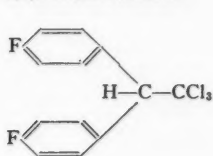
A. ISOMERS

I. DDT: 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane

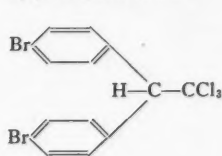
II. *o,p*-DDT: 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethane

B. ANALOGUES

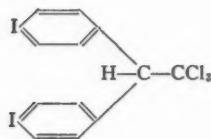
III. FLUORO-DDT: 1,1-bis(4-fluorophenyl)-2,2,2-trichloroethane



IV. BROMO-DDT: 1,1-bis(4-bromophenyl)-2,2,2-trichloroethane

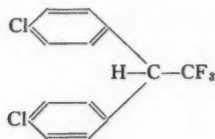


V. IODO-DDT: 1,1-bis(4-iodophenyl)-2,2,2-trichloroethane



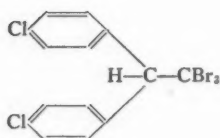
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VI. TRIFLUORO-DDT: 1,1-bis(4-chlorophenyl)-2,2,2-trifluoroethane



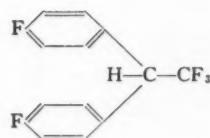
1/100

VII. TRIBROMO-DDT: 1,1-bis(4-chlorophenyl)-2,2,2-tribromoethane



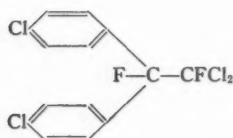
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VIII. FLUOROTRIFLUORO-DDT: 1,1-bis(4-fluorophenyl)-2,2,2-trifluoroethane



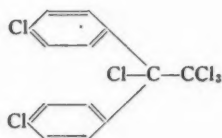
>1/100

IX. 1,1-bis(4-Chlorophenyl)-1-fluoro-2,2-dichloro-2-fluoroethane



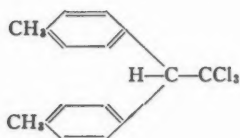
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X. 1,1-bis(4-Chlorophenyl)-1-chloro-2,2,2-trichloroethane



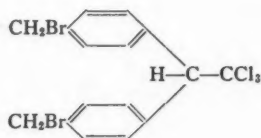
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XI. METHYL-DDT: 1,1-bis(4-methylphenyl)-2,2,2-trichloroethane



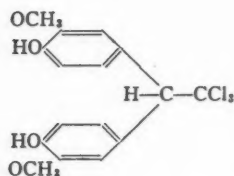
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XII. 1,1-bis(4-Bromomethylphenyl)-2,2,2-trichloroethane



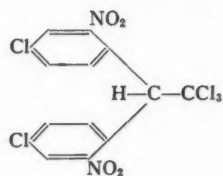
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XIII. 1,1-bis(3-Methoxy-4-hydroxyphenyl)-2,2,2-trichloroethane



0

XIV. 1,1-bis(2-Nitro-4-chlorophenyl)-2,2,2-trichloroethane

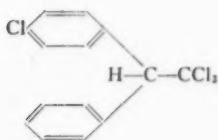


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C. REDUCED COMPOUNDS

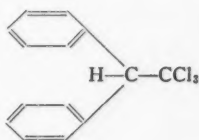
XV. MONOCHLOR-DDT: 1-phenyl-1-(4-chlorophenyl)-2,2,2-trichloroethane

>1/100



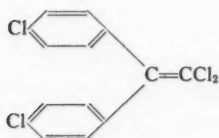
XVI. DT: 1,1-diphenyl-2,2,2-trichloroethane

0



XVII. ETHYLENE-DDT: 1,1-bis(4-chlorophenyl)-2,2-dichloroethylene

0



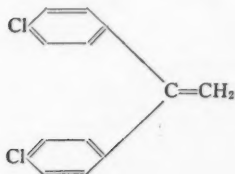
XVIII. BROMOETHYLENE-DDT: 1,1-bis(4-bromophenyl)-2,2-dichloroethylene

0



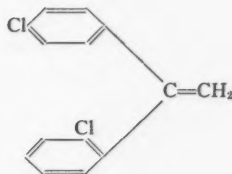
XIX. 1,1-bis(4-Chlorophenyl)-ethylene

>1/100



XX. 1-(2-Chlorophenyl)-1-(4-chlorophenyl)-ethylene

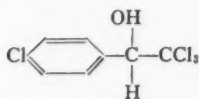
1/50



D. CARBINOLS, CARBINOL ANALOGUES, CARBINOL ESTERS

XXI. CARBINOL-DDT: 1-(4-chlorophenyl)-2,2,2-trichloroethanol

1/50

XXII. *levo*-1-(4-Chlorophenyl)-2,2,2-trichloroethanol

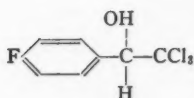
>1/50

XXIII. *dextro*-1-(4-Chlorophenyl)-2,2,2-trichloroethanol

<1/50

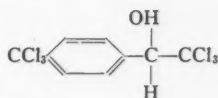
XXIV. 1-(4-Fluorophenyl)-2,2,2-trichloroethanol

1-2/5



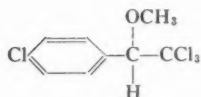
XXV. 1-(4-Trichloromethylphenyl)-2,2,2-trichloroethanol

0



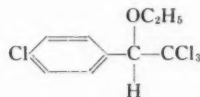
XXVI. 1-(4-Chlorophenyl)-1-methoxy-2,2,2-trichloroethane

1/50



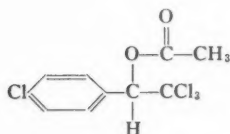
XXVII. 1-(4-Chlorophenyl)-1-ethoxy-2,2,2-trichloroethane

>1/75



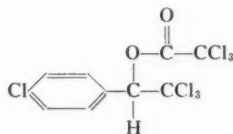
XXVIII. ACETOXY-CARBINOL-DDT: 1-(4-chlorophenyl)-2,2,2-trichloroethyl acetate

>1/10



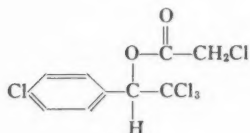
XXIX. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl trichloroacetate

0



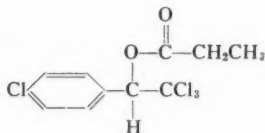
XXX. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl monochloroacetate

0



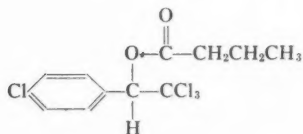
XXXI. PROPANOXY-CARBINOL-DDT: 1-(4-chlorophenyl)-2,2,2-trichloroethyl propionate

0



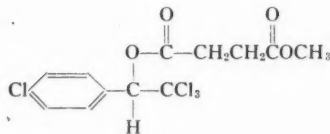
XXXII. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl butyrate

>1/500



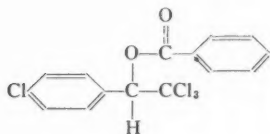
XXXIII. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl acetoxypropionate

0



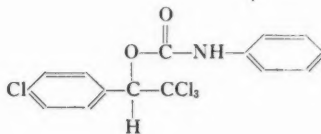
XXXIV. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl benzoate

0

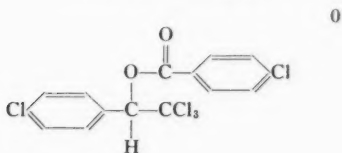


XXXV. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl benzamide

0

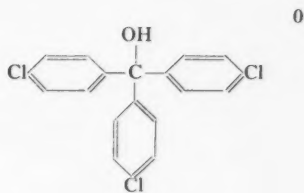


XXXVI. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl 4-chlorobenzoate



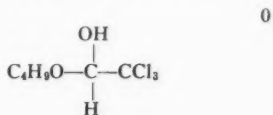
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XXXVII. Tri-(4-chlorophenyl)-methanol



0

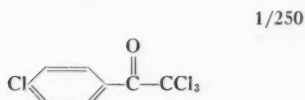
XXXVIII. 1-Butyl-2,2,2-trichloroethanol



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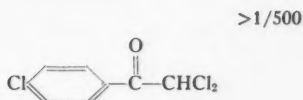
E. ETHANONES, ALDEHYDES, BENZOPHENONES, SULPHONES

XXXIX. 1-(4-Chlorophenyl)-2,2,2-trichloroethanone



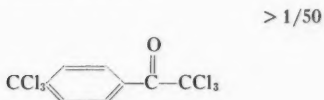
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XL. 1-(4-Chlorophenyl)-2,2-dichloroethanone



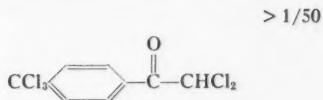
>1/500

XLI. 1-(4-Trichloromethylphenyl)-2,2,2-trichloroethanone



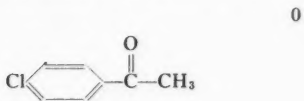
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XLII. 1-(4-Trichloromethylphenyl)-2,2-dichloroethanone



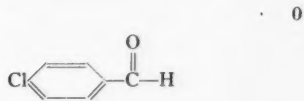
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XLIII. 1-(4-Chlorophenyl)-ethanone



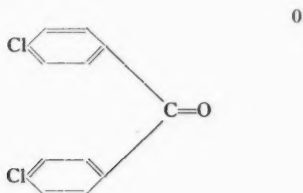
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XLIV. 4-Chlorobenzaldehyde

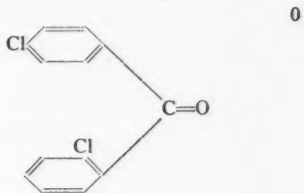


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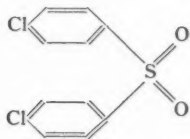
XLV. BENZOPHENONE: 4,4'-dichlorobenzophenone



0

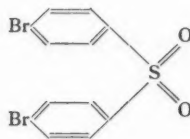
XLVI. *o,p*-Benzophenone: 2,4'-dichlorobenzophenone

0

XLVII. SULPHONE: 4,4'-dichlorophenyl
sulphone

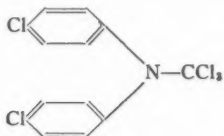
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XLVIII. 4,4'-Dibromophenyl sulphone



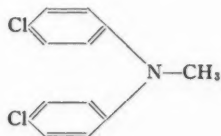
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F. DIPHENYLAMINES

XLIX. N-Trichloromethyl-4,4'-dichloro-
phenylamine

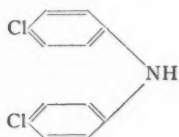
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L. N-Methyl-4,4'-dichlorophenylamine

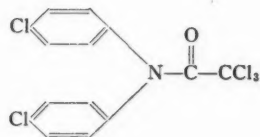


<1/500

LI. 4,4'-Dichlorophenylamine

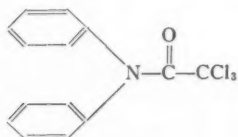


1/500

LII. N-Trichloroacetyl-4,4'-dichlorophenyl-
amine

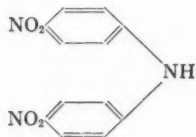
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LIII. N-Trichloroacetyldiphenylamine



0

LIV. 4,4'-Dinitrophenylamine



0

G. MISCELLANEOUS COMPOUNDS

LV. Hexachloroethane
 $\text{CCl}_3 \cdot \text{CCl}_3$

0

LVI. Pentachloroethane
 $\text{CCl}_3 \cdot \text{CCl}_2 \cdot \text{H}$

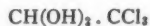
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LVII. 1-Butoxy-1-chloro-2,2,2-trichloroethane



1/500

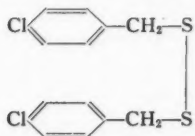
LVIII. Chloral hydrate



0

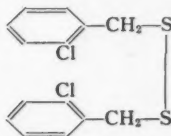
LIX. 4,4'-Dichlorodibenzyl disulphide

0



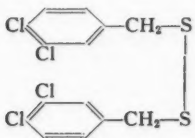
LX. 2,2'-Dichlorodibenzyl disulphide

0



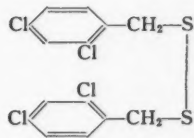
LXI. 3,4,3',4'-Tetrachlorodibenzyl disulphide

0



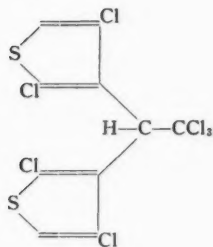
LXII. 2,4,2',4'-Tetrachlorodibenzyl disulphide

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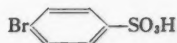
LXIII. 1,1-bis(2,5-Dichlorothieryl)-2,2,2-trichloroethane

0



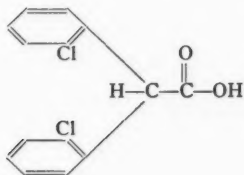
LXIV. 4-Bromobenzenesulphonic acid

<1/500



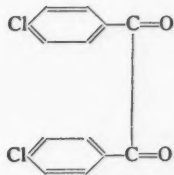
LXV. 1,1-bis(2-Chlorophenyl)-acetic acid

0



LXVI. 4,4'-Dichlorodiphenylbenzil

0

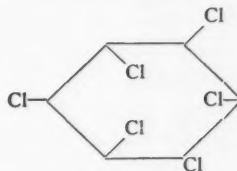


LXVII. 1,4-Dichlorobenzene

>1/250

LXVIII. GAMHEXANE: γ ,1,2,3,4,5,6-hexachlorocyclohexane

5



The standard 1 mgm. % of DDT gave a mortality varying on different occasions from 12 to 74%. Ideally comparisons should be made between concentrations of DDT and of the compounds that give a 50% mortality, i.e. the LD_{50} . However, when the mortality for a standard of 1 mgm. % was about 15%, the 50% mortality level would lie around 2.5 mgm. %; and when about 38% at approximately 1.5 mgm. % (Table I, DDT). Thus the factor of discrepancy between the relative potencies expressed in Table I and those that would have been obtained from the 50% mortality level is relatively small unless the dosage-mortality curves for DDT and the compound in question are very different in slope.

Morrison (17) says that the results of comparative insecticidal tests are largely a function of the methods used. In Table I two figures are shown that illustrate this contention. In the first test of monochlor-DDT (XV) impregnated paper rectangles were used as the test surface (18), instead of the glass of the test containers, and the standard concentration of DDT was 2.5 mgm. % instead of 1 mgm. %. By this method the relative potency of monochlor-DDT was 1/10th of DDT rather than the figure of 1/50 to 1/100th derived from later tests using the usual technique. Again, in the first test of carbinol-DDT the standard concentration of DDT was 2.5 mgm. % instead of 1 mgm. %. The relative potency was 1/30th rather than 1/50th. However, in view of the great variability between tests when the same technique was used, it is impossible to state how much of the difference between the results of the two methods is due to the difference in technique and how much is due to random variation.

Tests of gamhexane (hexachlorocyclohexane—Compound LXVIII) included very low concentrations and the results are expressed in Table II. The relative potency was approximately 5.

TABLE II
PERCENTAGE MORTALITIES AMONG *Drosophila melanogaster* ON EXPOSURE TO DDT AND GAMHEXANE

No. of flies per test	Percentage mortalities with concentration in mgm. %										
	DDT	Gamhexane									
	1	0.1	0.2	0.25	0.4	0.5	0.6	1	2.5	25	100
500	39							75	91	100	100
1000	39	11		47		81		82			
1000	22	8	18								
1000	24		5		32						
1000	41			27	69		77				
1500	35		43								

A comparison was made of the speeds of action of gamhexane, carbinol-DDT, and DDT. Tests were set up in the usual manner but mortality counts were made at the end of six hours as well as after the usual 18 hr. The

figures in Table III show that both gamhexane and carbinol-DDT cause death far more rapidly than DDT at concentrations that produce comparable mortalities after 18 hr. (DDT 2.5 mgm. %; carbinol-DDT 100 mgm. %; gamhexane 1 mgm. %.)

TABLE III

PERCENTAGE MORTALITIES AMONG 500 *Drosophila melanogaster* ON EXPOSURE TO DDT, CARBINOL-DDT, AND GAMHEXANE FOR 6 AND 18 HR.

Compound	Percentage mortalities with conc. in mgm. %						Hours of exposure
	1	2.5	25	100	250	500	
I. DDT	2	6	7	63			6
XXI. Carbinol				63	90	97	6
LXVIII. Gamhexane	60	90	100	100			6
I. DDT	39	79	82	99			18
XXI. Carbinol				91	99	100	18
LXVIII. Gamhexane	75	91	100	100			18

A number of compounds (*o,p*-DDT, trifluoro-DDT, methyl-DDT, carbinol-DDT, acetoxy-carbinol-DDT, propanoxy-carbinol-DDT, and 1-(4-chlorophenyl)-2,2-dichloroethanone) were tested for synergism. A solution of 0.2 mgm. of compound and 0.6 mgm. of DDT in 100 cc. of acetone was used for the preparation of test containers. The percentage mortalities after 18 hr. exposure of 1000 flies were compared to those from 0.6, 0.75, and 0.9 mgm. % of pure DDT. No synergism was found.

The same type of test was made with a mixture of 0.1 mgm. % of gamhexane and 0.6 mgm. % of DDT. The percentage mortalities were compared with those produced by the same compounds used independently. Separately gamhexane and DDT gave 15 and 22% mortality respectively; as a mixture they gave 50% mortality. Further tests would be necessary to decide whether this indicates a synergistic effect or merely a random variation.

Mammalian Toxicity

Preliminary tests for mammalian toxicity were done on a number of compounds. Each was dissolved in a minimum amount of olive oil and administered by forced feeding to four mice. The average weight of the animals was 25 gm. As an initial dose, 5 mgm. of compound was given, since neurotoxic symptoms were induced in all animals by DDT at this level. On five subsequent days 10 mgm. was given. The animals were observed through the period of treatment and for five days thereafter.

Table IV shows the exhibition of neurotoxic symptoms (tremors and hyperexcitability) and an arbitrary classification of toxicity; "high" indicates that three or four mice died; "medium" that one or two mice died; and "low" that none died. In addition to those compounds listed in the table groups of four mice received 25 mgm. only of acetoxy-carbinol-DDT and of sulphone; and

TABLE IV

INSECTICIDAL POTENCIES AND MAMMALIAN TOXICITIES OF DDT AND RELATED COMPOUNDS

Compound	Relative insecticidal potency	Mammalian toxicity	Neurotoxic symptoms
I. DDT	1	High	XX
II. <i>o,p</i> -DDT	0	Low	—
III. Fluoro	1/2.5 – 1/5	High	XX
IV. Bromo	1/500	High	XX
V. Iodo	0	Medium	XX
VII. Tribromo	0	Low	—
VIII. Fluorotrifluoro	1/75 – 1/100	Low	—
IX. X.	0	Medium	—
XI. Methyl	1/100	High	—
XII. Bromomethyl	0	Low	—
XV. Monochlor	1/50 – 1/100	Medium	X
XVI. DT	0	Low	—
XVII. Ethylene	0	Medium	—
XXI. Carbinol	1/50	Medium	—
XXXI. Propanoxy-carbinol	0	Low	—
XL.	1/250 – 1/500	Low	—
XLV. Benzophenone	0	Low	—
XLVI. <i>o,p</i> -benzophenone	0	Low	—
LXVII.	0	Low	—
LXVIII. Gamhexane	5	High	—

XX = tremors and hyperexcitability in all animals.

X = faint tremors and mild hyperexcitability in one animal.

15 mgm. only of trifluoro-DDT and of bromoethylene-DDT. None of these compounds produced neurotoxic symptoms but the last named killed two of the animals.

Discussion

Relative Insecticidal Potency

In Table V are grouped the more interesting compounds with the findings from the present work and that of other authors. Two compounds, DDD (1,1-*bis*(4-chlorophenyl)-2,2-dichloroethane), and methoxy-DDT (1,1-*bis*(4-methoxyphenyl)-2,2,2-trichloroethane), are included although not the subject of the present work. Where available a figure for potency relative to that of DDT has been inserted; the comparative ratings are always relative to the entire findings of the author.

The methods of testing differed. For instance Busvine (3, 4, 5) usually applied the compounds in an oily spray, which may have facilitated penetration of the insect cuticle. Haller (11) and Prill (22) are usually referring to aquatic insect larvae where ingestion of the compound can occur. However, despite variations in the technique of testing and estimation there is good general agreement.

TABLE V

INSECTICIDAL POTENCIES OF VARIOUS COMPOUNDS RELATIVE TO DDT AS DERIVED FROM VARIOUS AUTHORS. (REFERENCE AND INSECT SPECIES:— *Br.*, PRESENT REPORT, *Drosophila*; *Ps.*, (23), *Drosophila*; *Bs.*, (3, 4, 5), *Cimex*, *Pediculus*; *Hr.*, (11), *Anopheles*; *Pr.*, (22), *Culex*; *Sr.*, (27), *Carpocapsa*; *Dz.*, (8), *Pediculus*, *Calliphora*, *Formica*, *Calandra*, *Ephesia*, *Tineola*; *Lr.*, (15), *Tineola*, *Anthrenus*, *Attagenus*; *Mr.*, (19), *Calliphora*, *Formica*, *Calandra*, *Trogium*, *Thaumetopoea*; *Ms.*, (20), *Calliphora*, *Tineola*.)

Compound	<i>Br.</i>	<i>Ps.</i>	<i>Bs.</i>	<i>Hr.</i>	<i>Pr.</i>	<i>Sr.</i>	<i>Dz.</i>	<i>Lr.</i>	<i>Mr.</i>	<i>Ms.</i>
I. DDT	Good 1	Good 1	Good 1	Good 1	Good 1	Good	Good	Good	Good	Good
II. <i>o,p</i> -DDT	Nil 0	Poor 1/145	Poor 1/20– 1/28	Nil 0			Poor		Fair	
III. Fluoro	Good 1/5– 2/5	Good 9	Fair 1/3– 1/10	Fair 1/2	Good 1		Good		Good	
IV. Bromo	Poor 1/500	Fair 1/26	Good 1/2– 1/3	Good 1	Fair 1/5	Good		Fair	Fair	
V. Iodo	Nil 0	Nil 0			Fair 1/5					
VII. Tribromo	Poor 1/500			Poor 1/10				Good		Good
XI. Methyl	Fair 1/100	Fair 1/10	Fair 1/6– 1/7	Fair 1/2	Good 1	Good	Fair		Good	
XV. Monochlor	Fair 1/100	Poor 1/90	Fair 1/8	Fair 1/2	Fair 1/5				Good	
XVI. DT	Nil 0	Nil 0	Poor 1/25	Nil 0	Poor 1/10– 1/20	Poor	Poor		Fair	
XVII. Ethylene	Nil 0		Poor 1/20– 1/30		Fair 1/5	Nil	Poor		Fair	Poor
XXI. Carbinol	Fair 1/50	Fair 1/25	Poor 1/20				Good			
DDD		Fair 1/17	Good 1/2– 1/6	Good 1			Good	Fair	Good	Good
Methoxy			Good 1/2– 1/3	Good	Good 1			Good	Good	
LXVIII. Gamhexane	Good 5	Good 20	Good 18–20	Good 9						

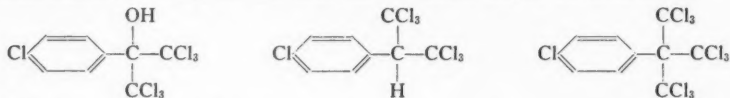
The ortho-para isomer of DDT was totally inactive in the present tests although some workers ascribe "poor" action to it. This compound was tested both after repeated recrystallization from organic solvents and after synthesis from orthocarbinol-DDT and chlorobenzene to eliminate contamination with DDT itself (25). Such contamination could be responsible for the above-mentioned activity. This isomer has been reported as the chief impurity in technical DDT (9, 12).

Fluoro-DDT has been found to be active by all workers but there is a big range in the comparative potencies expressed. Busvine (4) and Haller (11) both give it as less effective than the bromine analogue. Proverbs and Morrison (23) call attention to its volatility and this property may account for differing estimates. Bromo-DDT is listed as equitoxic with DDT by Haller but the figure is derived from comparisons where the weakest concentration of DDT already produced a 100% mortality.

Carbinol-DDT was rated as "fair" in the present work and by Proverbs and Morrison (23), but as poor by Busvine (4). Domenjoz (8) found it as active as DDT against the louse, fly, weevil, and ant but inactive against the clothes moth larva. Present tests included the optically active forms (31). *Levo*-carbinol was more active and *dextro*-carbinol less active than the racemic mixture (Compounds XXII and XXIII, Table I).

Carbinol-DDT was especially interesting in that it acted more rapidly than DDT and appeared to have a different mode of action. The dosage/mortality-probit line was appreciably steeper than that of DDT. Poisoned fruit flies did not show convulsive movements but died as though narcotized. The dead insects had their wings folded in the normal horizontal position and did not appear desiccated in contrast to the flies killed by DDT, which had vertically outstretched wings and shrivelled bodies. So closely did the dead insects resemble those that were merely anesthetized with ether that, in one test, they were removed from the test containers and kept under observation. They did not recover.

Carbinol-DDT is also interesting on account of its chemophysical properties. Despite the replacement of one para-substituted phenyl ring by a hydroxyl group it has an appreciable potency relative to that obtained through loss of the para substituent (monochlor-DDT). It has a low melting point and a water-solubilizing hydroxyl group (although Domenjoz (8) found it to be nearly three times as soluble in olive oil as DDT.) The first of a series of esters, acetoxycarbinol-DDT, showed a sharp rise in potency to 1/10th that of DDT and its mode of action seemed to be the same as that of the parent carbinol. Replacement of the hydroxyl group with methoxy and ethoxy groups (Compounds XXVI and XXVII) produced substances with "fair" activity although slightly less potent than the parent compound. Replacement of the para chlorine by fluorine gave a highly active compound, paralleling the same replacement in DDT itself. The activity of derivatives with one or two trichloromethyl groups on the first carbon atom might be interesting:—



Chemical Structure and Toxicity

Of the compounds tested, 16 had relative potencies of from 1/100th to 2/5ths that of DDT (Table I). The basic structure of their molecules was a two-carbon chain with a para-substituted phenyl ring on carbon 1 and a di- or

tri-halogen group on a saturated carbon 2. The only exceptions were the chlorophenylethylenes (Compounds XIX and XX) where the carbon chain was unsaturated.



This basic structure is represented by the above skeleton formulae where X may be F, Cl, CH₃, or (sometimes) CCl₃; where Y may be C₆H₄Cl, C₆H₅, OH, OCH₃, OC₂H₅, or O.CO.CH₃; and where Z may be F or Cl. These findings are similar to those of von Oettingen and Sharpless (21) and of Smith, Bauer, Stohlman, and Lillie (29) for mammalian toxicities but there are well marked exceptions.

Table VI shows the simplified findings available from four authors for the olive oil solubility, ease of dehydrochlorination, and mammalian toxicity of a series of the compounds. Insecticidal (and mammalian) toxicities have been inserted from the present work; where possible, to give values uncomplicated by other than unaided contact action.

The table shows that compounds with high insecticidal activity give neurotoxic symptoms in mammals but do not necessarily have high mammalian toxicity (DDT, fluor-DDT, methoxy-DDT, DDD—the latter is a fair insecticide only). Conversely compounds with high mammalian toxicity are not necessarily insecticidally active (bromo- and iodo-DDT). *o,p*-DDT, tribromo-DDT, DT, ethylene-DDT, and sulphone have both low mammalian and insecticidal toxicity. Carbinol-DDT, methyl-DDT, and monochlor-DDT have fair insecticidal activity and low mammalian toxicity, producing no neurotoxic symptoms.

These correlations are thought by some of us (H.C.B., M.D.) to suggest that the mechanism of toxic action may be similar for insect and mammal and that the differences are due to the manner of entry into the tissues. Savit, Kollros, and Tobias (26, 30) showed that the LD₅₀ to insects for both DDT and gamhexane was of the same order whether administered externally or internally. This is in complete contrast with the conditions in mammals where unaided percutaneous absorption is negligible. In insects the effective compounds must have an ability to penetrate the integument, a layer designed to hinder the passage of substances in either direction. From the integument it may pass into the hypodermis and thence to the hemolymph or (24) enter the lipid nerve sheath directly.

Läuger, Martin, and Müller (15) suggested that fat solubility of contact insecticides promoted entry. Martin and Wain (16) made the same assumption, adding a suggestion that toxic action itself was due to the liberation of hydrochloric acid at the vital centers. In Table VI four compounds are less fat soluble than DDT; bromo-DDT, iodo-DDT, tribromo-DDT, and sulphone. The first two are highly toxic to mammals but inert insecticidally; the last two are low in toxicity to both groups of animals. Conversely it

TABLE VI
INSECTICIDAL TOXICITY, OLIVE OIL SOLUBILITY, EASE OF DEHYDROCHLORINATION, AND MAMMALIAN TOXICITY OF VARIOUS COMPOUNDS
RELATED TO DDT

(O., VON OETTINGEN AND SHARPLESS (21); D., DOMENJOS (8); B., BUSVINE (3, 4, 5); M., MÜLLER (19); Br., PRESENT WORK

Compound	Insect toxicity		Olive oil solubility		Ease of dehydrochlorination			Mammalian toxicity			Neurotoxic symptoms					
	Br.		O.	D.	B.	O.	D.	M.	O.	D.	S.	Br.	O.	D.	S.	Br.
I. DDT	Good		1.0	1.0	1.0	1.0	1.0	1.0	1	1	1	High	XX	XX	XX	XX
II. <i>o,p</i> -DDT	Nil			1.1	2.5	0.2	0.1	0.1	>5	8		Low	—	—	—	—
III. Fluoro	Good	>4.0				0.2	0.6	1.0	5	1		High	X	XX	XX	XX
IV. Bromo	Poor	0.2				1.5		1.0	1		1	High	XX	XX	XX	XX
V. Iodo	Nil	0.0				1.5			2			Med.	XX			XX
VII. Tribromo	Poor	0.2				10.0			4			Low	—	—	—	—
XI. Methyl	Fair	1.2	0.8		2.0	0.0	0.0	0.3	>3	8		Low	—	—	—	—
XV. Monochlor	Fair	2.8				0.2			>5			Med.	—	—	—	—
XVI. DT	Nil	3.4	2.7	3.0		0.2	0.1	0.7	>4	5	14	Low	—	XX	—	—
XVII. Ethylene	Nil	2.0	0.9	2.0		0.0	0.0		>3	3	7	Med.	—	—	X	—
XXI. Carbinol	Fair		2.8							8		Med.	—	—	—	—
XLVII. Sulphone	Nil		0.1							60			—	—	—	—
DDD	Fair*	0.8	0.8		1.0	0.3	0.8	1.0	>5	15	20		—	XX	X	—
Methoxy	Good**	1.0	0.5	1.0		0.0	0.0	0.3	>4	5	50		—	XX	XX	—

* Proverbs and Morrison (23).

** Table V.

Note: X = mild or slight tremors.

XX = "DDT" tremors.

might be expected that some of the compounds with low mammalian toxicity are fair insecticides owing to compensatory high fat solubility (monochlor-DDT, carbinol-DDT, methoxy-DDT, and DDD). None of the six compounds showing fair or greater insecticidal activity had a low oil solubility. Solubilities of the compounds in substances more closely related to the lipids of insect integument than a vegetable oil might be more revealing. No correlation is seen between ease of dehydrochlorination and toxicity.

Latüger, Martin, and Müller (15) considered that the trihalogen group was fat-solubilizing and the para-chlorophenyl rings toxic. Martin and Wain (16) offered the reverse concept. Kirkwood and Phillips (14), with evidence from fluoro-DDT and trifluoro-DDT, supported the first theory. Tribromo-DDT has a very low fat solubility (21). Conversely, the halogen para phenyl substituents decrease fat solubility in the order fluorine, chlorine, bromine, and iodine.

This attribution of specific properties to specific parts of the molecule is unjustified. The removal of substituents (monochlor-DDT and DT) or even the removal of an entire chlorophenyl ring (carbinol-DDT) increases fat solubility. When considering the reactions of functional groups, the modifying influences of the rest of the molecule should not be forgotten. This is especially important in biological systems. The replacement of a single hydrogen atom by chlorine (Compound X), without affecting either the chlorophenyl or trichlorethane groups abolishes all activity. Conspicuous among all compounds is the ortho-para isomer of DDT. Merely by a shift in position of one chlorine atom all insecticidal action is lost and mammalian toxicity much reduced. The 2,2'-isomer has also been found inactive (6, 19) but the 3,4'-isomer was a good insecticide (7, 19). Similar findings are reported for the isomers of hexachlorocyclohexane of which gamhexane is but one (28). Internal energy relations of components of a molecule and allied steric factors are equal in importance with the actual nature of the constituent atoms.

Acknowledgment

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References

1. BARRY, G. T. Synthesis of DDT homologs. In preparation.
2. BROWNING, H. C., FRASER, F. C., SHAPIRO, S. K., GLICKMAN, I., and DUBRÔLE, M. The biological assay of DDT (1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane). In preparation.
3. BUSVINE, J. R. Insecticidal action of DDT. *Nature*, 156 : 169-170. 1945.
4. BUSVINE, J. R. Insecticidal tests with analogues of DDT. *J. Soc. Chem. Ind.* 65 : 356-360. 1946.
5. BUSVINE, J. R. New synthetic contact insecticides. *Nature*, 158 : 22. 1946.
6. CRISTOL, S. J., HALLER, H. L., and LINDQUIST, A. W. Toxicity of DDT isomers to some insects affecting man. *Science*, 104 : 343-344. 1946.
7. DEONIER, C. C. and JONES, H. A. TDE, 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)-ethane, as an anopheline larvicide. *Science*, 103 : 13-14. 1946.

8. DOMENJOZ, R. Action biologique de quelques dérivés du DDT. *Helv. Chim. Acta*, 29 : 1317-1322. 1946.
9. FORREST, J., STEPHENSON, O., and WATERS, W. A. Chemical investigations of the insecticide "DDT" and its analogues. Part I. Reactions of "DDT" and associated compounds. *J. Chem. Soc.* : 333-339. 1946.
10. GINSBURG, J. M. Comparative toxicity of DDT isomers and related compounds to mosquito larvae and fish. *Science*, 105 : 233-234. 1947.
11. HALLER, H. L. Wartime development of insecticides. *Ind. Eng. Chem.* 39 : 467-473. 1947.
12. HALLER, H. L., BARTLETT, P. D., DRAKE, N. L., NEWMAN, M. S., CRISTOL, S. J., EAKER, C. M., HAYES, R. A., KILMER, G. W., MAGERHEIN, B., MUELLER, G. P., SCHNEIDER, A., and WHEATLEY, W. The chemical composition of technical DDT. *J. Am. Chem. Soc.* 67 : 1591-1602. 1945.
13. KIRKWOOD, S. and DACEY, J. R. Synthesis of some analogues of 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT). I. Three fluorine analogues. *Can. J. Research*, B, 24 : 69-72. 1946.
14. KIRKWOOD, S. and PHILLIPS, P. H. The relationship between the lipid affinity and the insecticidal action of 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane and related substances. *J. Pharmacol.* 87 : 375-381. 1946.
15. LÄUGER, P., MARTIN, H., and MÜLLER, P. Über Konstitution und toxische Wirkung von natürlichen und neuen synthetischen insektentötenden Stoffen. *Helv. Chim. Acta*, 27 : 892-928. 1944.
16. MARTIN, H. and WAIN, R. L. Insecticidal action of DDT. *Nature*, 154 : 512-513. 1944.
17. MORRISON, F. O. The standardizing of a laboratory method for comparing the toxicity of contact insecticides. *Can. J. Research*, D, 21 : 35-75. 1943.
18. MORRISON, F. O. Comparing the toxicity of synthetic organic compounds. *Ann. Rept. Entomol. Soc. Ontario* : 18-20. 1946.
19. MÜLLER, P. Über Zusammenhänge zwischen Konstitution und insektizider Wirkung I. *Helv. Chim. Acta*, 29 : 1560-1580. 1946.
20. MYLIUS, A. and KOECHLIN, H. Berichtigungen zur Arbeit von P. Läger, H. Martin, und P. Müller "Über die Konstitution und toxische Wirkung von natürlichen und neuen synthetischen insektentötenden Stoffen". *Helv. Chim. Acta*, 29 : 405-411. 1946.
21. OETTINGEN, W. F. von and SHARPLESS, N. E. The toxicity and toxic manifestations of 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) as influenced by chemical changes in the molecule. *J. Pharmacol.* 88 : 400-413. 1946.
22. PRILL, E. A., SYNERHOLM, M. E., and HARTZELL, A. Some compounds related to the insecticide "DDT" and their effectiveness against mosquito larvae and houseflies. *Contr. Boyce Thompson Inst.* 14 : 341-353. 1946.
23. PROVERBS, M. D. and MORRISON, F. O. The relative insecticidal activities of DDT and related organic molecules. *Can. J. Research*, D, 25 : 12-44. 1947.
24. RICHARDS, A. G. Lipoid nerve sheaths in insects and their probable relation to insecticidal action. *J. New York Entomol. Soc.* 51 : 55-69. 1943.
25. ROSS, J. H., BOYER, R., GARMAISE, D. L., BARRY, G. T., and WHITE, H. L. Progress reports to the National Research Council, Canada. Unpublished. 1944-1946.
26. SAVIT, J., KOLLROS, J. J., and TOBIAS, J. M. Measured dose of gamma hexachlorocyclohexane (γ 666) required to kill flies and cockroaches and a comparison with DDT. *Proc. Soc. Exptl. Biol. Med.* 62 : 44-48. 1946.
27. SIEGLER, E. H. and GERTLER, S. I. Toxicity of diaryl trichloroethanes and dichloroethylenes to codling moth. *J. Econ. Entomol.* 37 : 845. 1944.
28. SLADE, R. E. The γ -isomer of hexachlorocyclohexane (gammexane). *Chem. Ind.* 314-319. 1945.
29. SMITH, M. I., BAUER, H., STOHLMAN, E. F., and LILLIE, R. D. The pharmacologic action of certain analogues and derivatives of DDT. *J. Pharmacol.* 88 : 359-365. 1946.
30. TOBIAS, J. M., KOLLROS, J. J., and SAVIT, J. Relation of absorbability to the comparative toxicity of DDT for insects and mammals. *J. Pharmacol.* 86 : 287-293. 1946.
31. WHITE, H. L. Synthesis of DDT homologs. In preparation.

THE INSECTICIDAL ACTIVITY OF DDT AND RELATED COMPOUNDS AGAINST DIFFERENT INSECT SPECIES¹

BY H. C. BROWNING,² S. K. SHAPIRO,³ AND M. DUBRÛLE⁴

Abstract

DDT and its fluoro- and methyl-analogues, its carbinol and carbinol acetate, and monochlor-DDT (1-(4-chlorophenyl)-1-phenyl-2,2,2-trichloroethane) were tested for contact insecticidal activity against two beetles (*Calandra granaria* and *Tribolium confusum*), a moth (*Ephestia kuhniella*), a plant bug (*Oncopeltus fasciatus*), and a cockroach (*Blatella germanica*).

Fluoro-DDT showed specificity against the cockroach and carbinol-DDT against the plant bug. Comparisons with the results of earlier testing against *Drosophila melanogaster* justified its use as an indicator species.

Introduction

A large series of compounds related to DDT had been tested against *Drosophila melanogaster* (2) in co-ordination with a program of synthesis in the Department of Chemistry, McGill University (1, 14, 19) and the National Research Council Laboratories (9). At the conclusion of this program it was felt desirable to examine selected compounds for potency against other insects, partly to detect specific activities and partly to examine the validity of using *Drosophila melanogaster* as an indicator of insecticidal efficiency.

In the previous work compounds were arbitrarily classified as having good activity if the relative potency (compared to DDT) was 1/10th or more; as fair if from 1/50th to 1/100th; and as poor if from 1/250th to 1/500th. Five compounds were available, as was DDT, in pure crystalline form. The activity of two was good and of the other three, fair. These compounds, their synonyms and relative potencies against *Drosophila* are listed on p. 302.

Methods

Each compound was tested against five insect species: the grain weevil, *Calandra granaria*; the flour beetle, *Tribolium confusum*; the flour moth, *Ephestia kuhniella*; the large milkweed bug, *Oncopeltus fasciatus*; and the German cockroach, *Blatella germanica*.

The grain weevils and flour beetles were cultured in wheat and flour respectively. Each culture was cleared of all adults every 10 days so that these insects, used for testing, were from 0 to 10 days old from emergence. The flour moth was cultured on untreated peanuts and all adults removed every week

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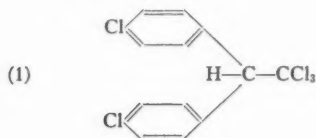
Contribution from the Department of Genetics, McGill University, Montreal, Que., with financial assistance from the National Research Council of Canada.

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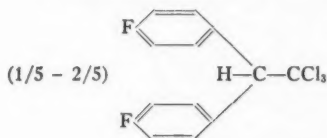
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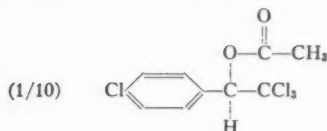
A. DDT: 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane



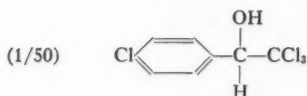
B. FLUORO-DDT: 1,1-bis(4-fluorophenyl)-2,2,2-trichloroethane



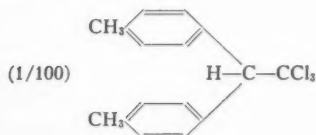
C. ACETOXY-CARBINOL-DDT: 1-(4-chlorophenyl)-2,2,2-trichloroethyl acetate



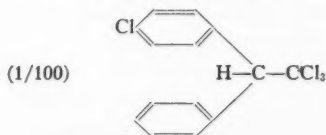
D. CARBINOL-DDT: 1-(4-chlorophenyl)-2,2,2-trichloroethanol



E. METHYL-DDT: 1,1-bis(4-methylphenyl)-2,2,2-trichloroethane



F. MONOCHLOR-DDT: 1-(4-chlorophenyl)-1-phenyl-2,2,2-trichloroethane



for testing. The milkweed bugs were reared on milkweed seed and egg masses removed at biweekly intervals; these eggs were observed for hatching and the nymphs were used when from four to seven days old. Cocoon-bearing cockroaches were cultured on fox chow; the nymphs were used 14 days after emergence from the cocoons. No attempt was made to determine the exact instar of milkweed bugs or cockroaches.

For all species except *Blatella germanica* and for all compounds including DDT itself, test surfaces were prepared by wetting the internal surfaces of half-pint milk bottles with acetone solutions at appropriate strengths. The bottles were allowed to dry at room temperature for four to five hours. From 10 to 40 insects were placed in each bottle. The bottles were stoppered with cotton wool plugs moistened in 5% aqueous molasses solution and incubated at 30° C. The number of insects per bottle, the number of bottles, and the period of incubation varied with the species. All compounds were tested against a single species at the same time. Mortality counts were made at the end of the incubation period. The testing method was essentially that used in earlier work (2) for *Drosophila*.

For the cockroach a different method was used. Approximately 50 mgm. of DDT or compound (as a finely divided powder) was placed at the bottom of half-pint milk bottles and the requisite number of roaches added. The bottles were stoppered and incubated as for the other species. The incubation period was 48 hr.

Results

Preliminary tests showed that the preparation of test bottles with a solution of DDT containing 2.5 mgm. per 100 cc. of acetone would give a percentage mortality above 30 and below 80 for all insects except *Blatella germanica*, providing that the time of incubation was adjusted accordingly. This concentration was taken as the standard for comparison. *Calandra granaria* and *Tribolium confusum* required five and seven days' incubation respectively and *Oncopeltus fasciatus* and *Ephestia kuhniella* 40 hr. The related compounds were used at strengths equivalent in insecticidal potency to that of the standard as based on results with *Drosophila* i.e., fluoro-DDT, 2.5×5 mgm. %; acetoxy-carbinol-DDT, 2.5×10 mgm. %; carbinol-DDT, 2.5×50 mgm. %; and methyl-DDT and monochlor-DDT, 2.5×100 mgm. %. At such a concentration the carbinol produced a 100% mortality against *Oncopeltus fasciatus*. The test was repeated for this insect using the carbinol at 50 mgm.%, 5 mgm. %, and 2.5 mgm. %. The test was also repeated for the fluorine analogue using the original concentration.

The same tests with *Blatella germanica* showed that this roach was not killed in a reasonable time even when DDT at a concentration of 50 mgm. % was used to prepare test bottles. Consequently this insect was exposed to pure DDT and pure compounds as already described.

The larvae of *Ephestia kuhniella* were tested in the same manner as the adults. There was no mortality and all the larvae pupated and emerged as adult moths.

Table I summarizes the results, giving average percentage mortalities for each compound against each species (except the larval *Ephestia kuhniella*). Control mortalities, in untreated bottles, were 20% for *Calandra granaria*, 6% for *Tribolium confusum*, and 0% for the other three species. Table II expresses the potencies relative to DDT of each compound to each species.

With each compound there were examples of anomalous action. The fluorine analogue was twice as effective as DDT against *Blatella*. The acetoxy-carbinol, inactive against *Oncopeltus*, *Ephestia*, and *Tribolium*, was relatively potent against *Blatella*. The carbinol was equal in potency with DDT against *Oncopeltus*. Methyl-DDT and monochlor-DDT, maintaining their moderate potency in general, were inactive against *Tribolium*.

Discussion

The findings with fluoro-DDT correspond with those of Domenjot (6) and Müller (10). Not only did they find that it maintained its high activity against all species tested but that it was more effective than DDT against *Calliphora*, *Calandra*, and *Formica*. Its specificity against the roach, as found in the present work, is interesting for the classical roach poison, sodium fluoride, has been shown to act as a contact rather than as a stomach insecticide (7). However, Busvine (3) found fluoro-DDT much less effective than DDT against *Pediculus* and *Cimex* and the results of Proverbs and Morrison (13) suggest that it decomposes or volatilizes readily.

TABLE I

PERCENTAGE MORTALITIES AMONG FIVE INSECT SPECIES ON EXPOSURE TO VARIOUS CONCENTRATIONS OF DDT AND RELATED COMPOUNDS

Compound	Conc. in mgm. %	Insect species and percentage mortalities with no. of insects per test and no. of tests				
		<i>Calandra granaria</i> 40 × 10	<i>Tribolium confusum</i> 40 × 5	<i>Ephestia kuhniella</i> 10 × 5	<i>Oncopeltus fasciatus</i> 40 × 8	<i>Blatella germanica</i> *
DDT	2.5	78	29	62	51	37
	2.5				58	
Fluoro-DDT	12.5	63	24	66	55	80
	12.5				49	
Acetoxy-carbinol- DDT	25.0	38	0	0	0	30
Carbinol-DDT	125.0	27	17	46	100	0
	50.0				100	
	5.0				76	
	2.5				52	
Methyl-DDT	250.0	52	0	68	42	17
Monochlor-DDT	250.0	73	0	60	31	7

* Tested at the same concentration of all compounds.

TABLE II

POTENCIES OF FIVE COMPOUNDS AGAINST DIFFERENT INSECT SPECIES RELATIVE TO DDT

Species	Fluoro- DDT	Acetoxy- carbinol- DDT	Carbinol- DDT	Methyl- DDT	Monochlor- DDT
<i>Drosophila</i> *	Good	Good	Fair	Fair	Fair
<i>Oncopeltus</i>	Good	Nil	Good	Fair	Fair
<i>Ephestia</i>	Good	Nil	Fair	Fair	Fair
<i>Calandra</i>	Good	Fair	Poor	Fair	Fair
<i>Tribolium</i>	Good	Nil	Poor	Nil	Nil
<i>Blatella</i>	Very good	Good	Nil	Fair	Poor

* See (2). Very good—relative potency more than 1; good—relative potency 1 to 1/10th; fair—relative potency 1/50th to 1/100th; poor—relative potency less than 1/100th; nil—no activity.

Carbinol-DDT was more effective than DDT against *Calliphora*, *Formica*, and *Pediculus* according to Domenjoz (6) but Busvine (3) does not agree with the findings towards the last-named species. Methyl-DDT has been found as potent as DDT against *Culex* (12), *Carpocapsa* (16), and *Calliphora* (10); so has monochlor-DDT against *Calliphora* (10). The carbinol acetate has not been examined by these authors.

Examples of inactivity of other compounds towards certain species are conspicuous throughout the work of Domenjoz (6) and of Müller (10). Haller (8) mentions that methyl-DDT and methoxy-DDT (1,1-bis(4-methoxyphenyl)-2,2,2-trichloroethane) have specificity against the codling moth and lack of it against the corn borer while DDD (1,1-bis(4-chlorophenyl)-2,2-dichloroethane) showed the reverse selectivity. Turner (18) found methoxy-DDT more effective than DDT against the Mexican bean beetle at one-eighth the concentration. Busvine (3, 4) found methoxy-DDT as effective against *Cimex* as DDT though only one-third as toxic to *Pediculus*.

The reasons for these specificities are likely to be found in the nature of the insect integument and the chemophysical properties of the insecticidal compound. The outermost layer of the integument consists of a mixture of fats and waxes and may be further covered with fatty films of secretion as it is in the cockroach (20). It has been shown that pyrethrins enter the integument faster if this lipoid is first removed (21). The inner layer varies in thickness and degree of impregnation with protein, chitin, fats, waxes, and phenols (5, 20), not only from place to place in the individual but even between closely related species (20). The importance of such variation is suggested by the fact that both the gamma isomer of hexachlorocyclohexane and DDT are much more toxic to newly emerged *Calliphora* than to the older individuals (15, 17); it is following on emergence that the integument undergoes chemical changes towards impermeability (20). The only pertinent information available on the present compounds is that they are all as or more oil soluble than DDT, the fluorine analogue especially so (6, 10, 11). A histochemical investigation of the integument of species differing in susceptibility to DDT and related compounds while subject to the contact action of these substances should be instructive.

The order of increasing susceptibility towards DDT was *Blatella*, *Tribolium*, *Calandra*, *Ephestia*, *Oncopeltus*, and *Drosophila*. The dipteran *Calliphora* was the most sensitive insect to the action of 20 compounds tested by Domenjoz (6) and to that of 37 tested by Müller (10). Certain compounds were inactive against one or more species in the present work although active against *Drosophila*. It appears that the use of this insect as a detector or indicator of insecticidal activity is a valid procedure.

Acknowledgment

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References

1. BARRY, G. T. Synthesis of DDT homologs. In preparation.
2. BROWNING, H. C., FRASER, F. C., SHAPIRO, S. K., GLICKMAN, I., and DUBRÔLE, M. The biological activity of DDT and related compounds. *Can. J. Research, D*, 26:282-300. 1948.
3. BUSVINE, J. R. Insecticidal tests with analogues of DDT. *J. Soc. Chem. Ind.* 65: 356-360. 1946.

4. BUSVINE, J. R. Insecticidal action of DDT. *Nature*, 156 : 169-170. 1945.
5. DENNEL, R. A study of insect cuticle: the formation of the puparium of *Sarcophaga falcata* Pand. (Diptera). *Proc. Roy. Soc. London, B*, 134 : 79-110. 1947.
6. DOMENJOZ, R. Action biologique de quelques dérivés du DDT. *Helv. Chim. Acta*, 29 : 1317-1322. 1946.
7. GRIFFITHS, J. T. and TAUBER, O. E. Evaluation of sodium fluoride as a stomach poison and as a contact insecticide against the roach, *Periplaneta americana* L. *J. Econ. Entomol.* 36 : 536-540. 1943.
8. HALLER, H. L. Wartime development of insecticides. *Ind. Eng. Chem.* 39 : 467-473. 1947.
9. KIRKWOOD, S. and DACEY, J. R. Synthesis of some analogs of 1,1-bis-(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT). I. Three fluorine analogs. *Can. J. Research, B*, 24 : 69-72. 1946.
10. MÜLLER, P. Über Zusammenhänge zwischen Konstitution und insektizider Wirkung I. *Helv. Chim. Acta*, 29 : 1560-1580. 1946.
11. OETTINGEN, W. F. VON and SHARPLESS, N. E. The toxicity and toxic manifestations of 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) as influenced by chemical changes in the molecule. *J. Pharmacol.* 88 : 400-413. 1946.
12. PRILL, E. A., SYNERHOLM, M. E., and HARTZELL, A. Some compounds related to the insecticide "DDT" and their effectiveness against mosquito larvae and houseflies. *Contr. Boyce Thompson Inst.* 14 : 341-353. 1946.
13. PROVERBS, M. D. and MORRISON, F. O. The relative insecticidal activities of DDT and related organic molecules. *Can. J. Research, D*, 25 : 12-44. 1947.
14. ROSS, J. H., BOYER, R., GARMAISE, D. L., BARRY, G. T., and WHITE, H. L. Progress reports to the National Research Council, Canada. Unpublished. 1944-46.
15. SAVIT, J., KOLLROS, J. J., and TOBIAS, J. M. Measured dose of gamma hexachlorocyclohexane (γ666) required to kill flies and cockroaches and a comparison with DDT. *Proc. Soc. Exptl. Biol. Med.* 62 : 44-48. 1946.
16. SIGLER, E. H. and GERTLER, S. I. Toxicity of diaryl trichloroethanes and dichloroethylenes to codling moth. *J. Econ. Entomol.* 37 : 845. 1944.
17. TOBIAS, J. M., KOLLROS, J. J., and SAVIT, J. Relation of absorbability to the comparative toxicity of DDT for insects and mammals. *J. Pharmacol.* 86 : 287-293. 1946.
18. TURNER, N. Organic insecticides for control of the Mexican bean beetle. *Connecticut Agr. Expt. Sta. Bull.* 511. In press. 1947.
19. WHITE, H. L. Synthesis of DDT homologs. In preparation.
20. WIGGLESWORTH, V. B. The principles of insect physiology. E. P. Dutton & Co., Inc., New York. 1939.
21. WIGGLESWORTH, V. B. Some notes on the integument of insects in relation to the entry of contact insecticides. *Bull. Entomol. Research*, 33 : 205-218. 1942.

ASCARIS LUMBRICOIDES INFECTION IN GUINEA PIGS WITH SPECIAL REFERENCE TO EOSINOPHILIA AND RESISTANCE¹

By A. MURRAY FALLIS²

Abstract

Clinical symptoms of *Ascaris* infection were produced in guinea pigs by feeding several thousand eggs. Such infections caused a temporary loss in weight and severe congestion of the lungs but no elevation in temperature was observed. An eosinophilia was associated with infection and it reached higher levels following repeated infections. Injections of antigen caused a temporary rise in the number of eosinophiles. Guinea pigs developed a resistance as a result of infection. Some resistance was retained for at least 15 weeks following infection. A slight passive resistance resulted from injections of large quantities of serum from resistant animals and from injections of a liver extract prepared from resistant animals. The resistance was apparent from the amount of congestion in the lungs and the number and size of the larvae recovered from the lungs. The eosinophiles per se were not responsible for the resistance observed. It appeared that the body defences, in resistant animals, acted against the parasites before they reached the liver and more especially before they reached the lungs.

Work, of several investigators, has shown that animals that have been infected with *Ascaris* spp. are resistant to subsequent infection with the same species. Opinions differ regarding the duration and nature of the resistance and quantitative measurements have not always been made.

Hadwen (6) postulated, from his study of ascariasis in horses, that "immunity to ascarids is stimulated and increased by repeated attacks of these parasites". He suggested that, in addition to other antiparasitic substances produced in the body to neutralize the cast off products of the worms, some substance is secreted by the eosinophiles that is detrimental to the worms. Morgan (9), in his work on *Ascaris* infection in hogs felt the evidence for an acquired immunity was far from conclusive. Wagner (15) showed, from experimental studies on mice infected with *Ascaris lumbricoides*, that fewer larvae were found in the livers and lungs of animals that had been infected previously. De Boer (4) found that hogs that had been infected previously were resistant to subsequent infection. Roberts (12) found that hogs that were exposed to continuous infection developed an immunity that he thought was concerned with the leucocytes, chiefly the eosinophiles, and the reticuloendothelial system. He thought the body defences were mobilized at the intestinal wall, liver, and lungs, especially at the intestine.

An extensive investigation of the cellular response in guinea pigs that had acquired resistance to infection with pig *Ascaris* was carried out by Kerr (8). He observed that a larger number of guinea pigs survived lethal doses of *Ascaris*

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eggs if they had received sublethal doses one week previously. He thought the resistance might be of short duration, lasting for only a short time after the larvae had disappeared from the body of the host. There was some indication also that the more sublethal infections a guinea pig received the more solid was the immunity. He noticed that the larvae did not grow as fast in the livers of resistant guinea pigs and there was less hemorrhage in the lungs because he thought fewer larvae had reached the lungs, and, moreover, they were being destroyed there.

The present investigation was begun in order to study (1) the effect of the parasite on the host, (2) the eosinophilia associated with infection, (3) the resistance that is produced by infection.

Materials and Methods

Guinea pigs of comparable sizes were selected for each experiment. *Ascaris* eggs, obtained by dissection from the distal portion of the uterus of mature female worms, were incubated in 0.5% formalin at 30° C. for at least three weeks. It was assumed that eggs were infective after this incubation.

The number of eggs fed each animal was determined by a dilution method or by counting the total number (when the dose contained fewer than one hundred eggs). The eggs were collected on small disks of fine filter paper, which were then placed in No. 5 gelatin capsules and fed to the guinea pigs. The capsules were swallowed more readily if softened with water and coated with sugar.

Blood smears and differential counts of 200 white blood cells were made in the usual manner.

Larvae were isolated from the tissues by digesting the latter in pepsin and hydrochloric acid at 37° C. Digestion of liver tissue was improved by comminution in the Waring Blendor (Fallis (5)) before adding the enzyme. Larvae were sometimes killed by digestion as Ransom and Cram (11) have pointed out. However, there was no evidence to show that they were digested to such an extent that they could not be recognized. Moreover the error should be similar in each experiment. The number of larvae remaining in the residue was counted in a Petri dish that had been ruled in squares. Larvae were measured following fixation in hot alcohol.

Ascaris antigen was prepared by drying whole specimens in a vacuum oven at 37° C., grinding them in a mortar, and suspending the product in phenolized saline. This antigen was stored in the refrigerator. Injections of antigen were given by the intraperitoneal route.

An extract was prepared as follows from the livers of a number of guinea pigs that were sacrificed 11 days after they were fed several thousand *Ascaris* eggs. The livers were removed and comminuted in a Waring Blendor with an equal volume of saline for two minutes. The resulting emulsion was

stirred continuously and fast frozen in a mixture of alcohol and solid carbon dioxide. It was then removed and thawed. The freezing and thawing were carried out three times. The mixture was then centrifuged and the supernatant was filtered through a Büchner filter. A volume of absolute alcohol was added to the filtrate to make the alcoholic concentration 80%. The addition of the alcohol produced a heavy precipitate that was allowed to settle for 16 hr. at room temperature. The precipitate was then thrown down by centrifuging and the supernatant was placed in a vacuum desiccator at 30° C. The supernatant was evaporated from 60 cc. to 20 cc. in 48 hr. A specific gravity determination of the remaining liquid indicated that the alcohol had been removed. Water was added to make the volume up to 30 cc. and the liquid was filtered through a Berkefeld candle. The filtrate was stored in the refrigerator. A liver extract was prepared at the same time, and in a similar way, from a group of guinea pigs that had not been infected.

Serum was collected from the blood of normal guinea pigs and from guinea pigs that had been infected. The so-called 'normal' serum was obtained from the blood of a group of noninfected guinea pigs. The 'resistant' serum was obtained from the blood of guinea pigs that had been infected three weeks previously by feeding each of them 20,000 to 30,000 *Ascaris* eggs. These sera were filtered through Berkefeld candles and stored in the refrigerator.

Rate of Growth, Temperature, and Eosinophile Response in Guinea Pigs Fed *Ascaris* Eggs

The rate of growth, temperature, and eosinophile level was followed in guinea pigs that received the following treatment: 10 *Ascaris* eggs daily for 14 days; 20 eggs daily for 14 days; single dose of several thousand eggs; no infection. There were five guinea pigs in each group.

No significant elevation in temperature was observed in any of the guinea pigs during the infection and for one week following the last dose of eggs. This observation differs from that of Roberts (12) who found an elevation in the temperature of hogs fed large doses of eggs over a prolonged period. Guinea pigs that received 10 or 20 eggs daily made similar gains in weight to those in the control group, which received no eggs. The percentage of eosinophiles in the blood was also similar with the exception of that in a single animal fed 20 eggs daily in which the eosinophiles increased to about 15% following infection. The guinea pigs that received a single dose of several thousand eggs began to lose weight four to five days following the ingestion of the eggs (Fig. 1) and at the same time there was an increase in the eosinophiles in the peripheral blood. Kerr (8) and Roberts (12) have shown also that an eosinophilia is associated with infection in guinea pigs. Those pigs that survived the infection began to gain weight about 10 days after they had been fed the *Ascaris* eggs (Fig. 1). There was a marked decline in the number of eosinophiles about 12 days after the ingestion of the eggs.

The lungs of one guinea pig that had been fed several thousand eggs, and that died eight days after infection, showed extensive congestion and a large number of eosinophiles (Fig. 2).

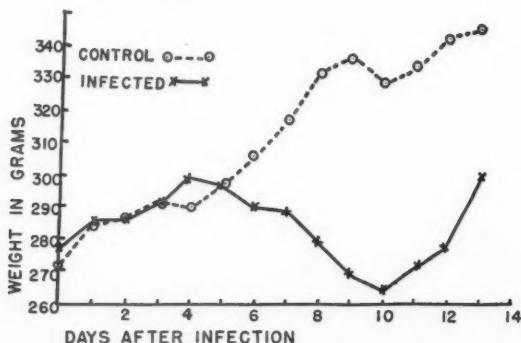


FIG. 1. Average rate of growth of four guinea pigs following infection by feeding several thousand eggs compared to that of five guinea pigs that received no eggs.

Rate of Migration of the Larvae Through the Body

Sixteen guinea pigs weighing approximately 300 gm. were each fed about 13,000 eggs. Two of the guinea pigs were sacrificed daily, beginning three days after infection, to determine the rate of migration of the larvae to the lungs.

The maximum number of larvae was recovered from the lungs of the guinea pigs eight days after they had ingested the eggs. Relatively few of the larvae reached the lungs before the fifth day following infection. The total number of larvae migrating to the lungs was small in comparison with the number of eggs fed.

Resistance in Guinea Pigs Infected Previously

Thirty guinea pigs were infected with *Ascaris* by feeding each pig over 9000 eggs. The guinea pigs showed a decline in weight, following infection, similar to that illustrated in Fig. 1. There was also a marked rise in the number of eosinophiles in the blood following infection. The number returned to the normal level two to three weeks later. One month after this infection each guinea pig was dosed again with approximately 27,000 eggs. At the same time each of a second group of 30 guinea pigs was infected for the first time by feeding a similar number of *Ascaris* eggs to that given to the animals in the first group. Five other guinea pigs were kept as controls and received no *Ascaris* eggs.

The average percentage of eosinophiles in the peripheral blood of the guinea pigs in these three groups on successive days following infection is shown in Fig. 3. The average is based on counts from more animals at the beginning of the experiment than at the end as three or four of the pigs in each group were sacrificed daily commencing five days after their final infection.

PLATE I

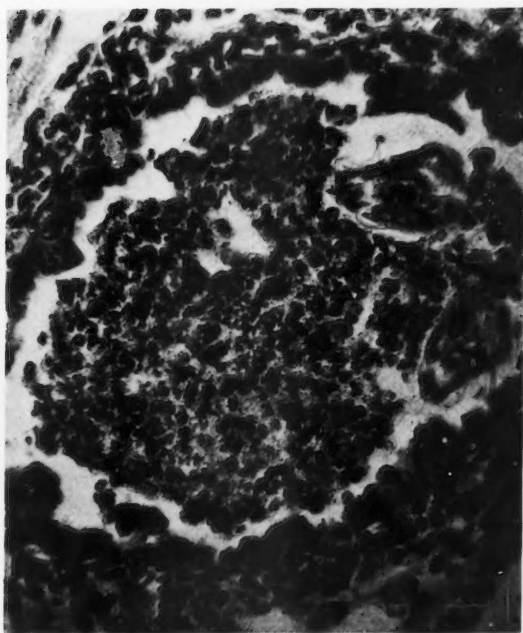
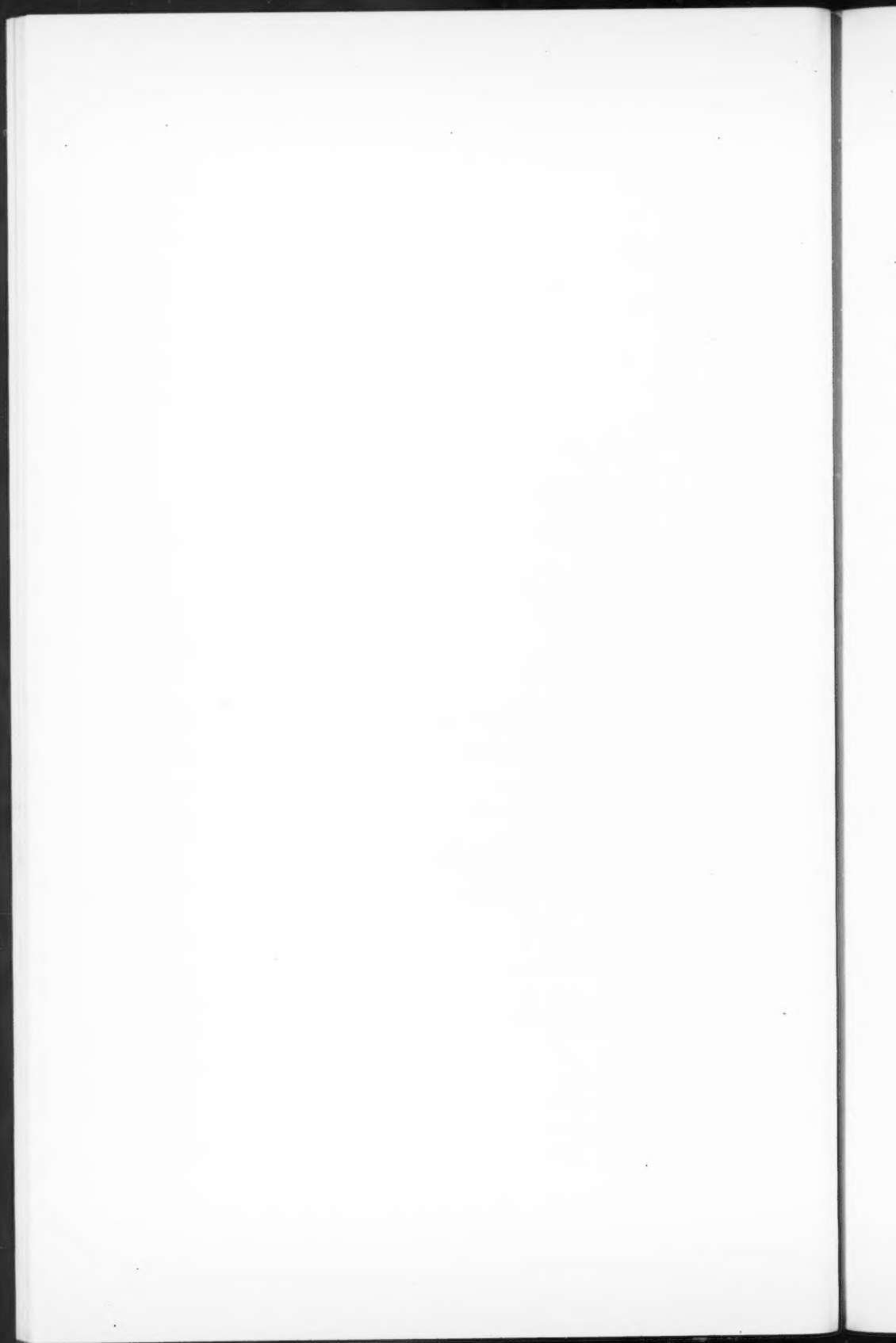


FIG. 2. Extensive eosinophilia in section of lung of guinea pig eight days after infection with *Ascaris lumbricoides*.



The gross appearance of the lungs of the guinea pigs in each group, with the exception of those from animals killed 5 and 10 days after infection, is illus-

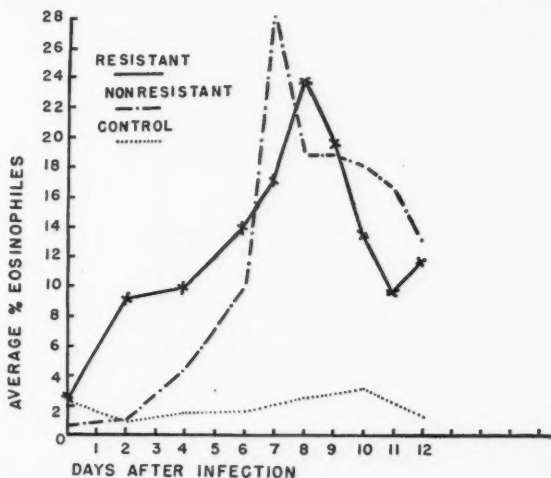


FIG. 3. Average percentage of eosinophiles in the blood of resistant and nonresistant guinea pigs following infection.

trated in kodachrome pictures (Fig. 4). The weight of the lung of each guinea pig was recorded also for comparison with the weight of the animal (Fig. 8).

The average number of larvae recovered from the lungs of the two groups of pigs on successive days following infection is illustrated in Fig. 9. It is apparent that fewer larvae reached the lungs of the pigs that had been infected only once, that is to say, guinea pigs infected with *Ascaris* are resistant to a second infection given one month after the first. In no instance, however, did the number of larvae recovered from the lungs nearly equal the number of eggs that had been fed. No explanation can be given for the recovery of fewer larvae from the guinea pigs in the nonresistant group on the seventh day than on either the sixth or eighth day after infection, but it will be observed (Fig. 4) that there was less congestion in the lungs of the guinea pigs killed on the seventh day.

The congestion in the lungs of the guinea pigs that had been infected for the first time was, in general, more marked than in those that had been infected more than once (Figs. 4 and 8) and was no doubt related to the number of larvae present (Fig. 9). It will be observed, from a comparison of Figs. 4 and 8, that the weights of the lungs of the nonresistant guinea pigs increased, in relation to their body weights, as the congestion in the lungs increased, and the weights of the lungs decreased as the congestion disappeared. The lungs from the resistant animals on the other hand were much lighter, in relation

TABLE I

AVERAGE SIZE *Ascaris* LARVAE IN LUNGS OF RESISTANT AND NONRESISTANT GUINEA PIGS ON DIFFERENT DAYS FOLLOWING INFECTION. THE FIGURES IN PARENTHESES INDICATE THE NUMBER OF MEASUREMENTS USED TO CALCULATE THE AVERAGES

Days after infection	Average size of larvae in mm.	
	Resistant	Nonresistant
5	0.4 (2)	0.53 (20)
6	0.55 (39)	0.88 (97)
7	0.89 (47)	1.1 (113)
8	1.1 (28)	1.3 (94)
9	1.2 (28)	1.4 (77)
10	1.3 (15)	1.5 (71)
11	1.3 (14)	1.4 (59)
12	1.5 (6)	1.5 (20)

to the weights of the animals, except on the fifth day after infection, which would seem to be related also to the extent of the congestion. It will be observed, however, that the lungs from some of the resistant animals, e.g. those sacrificed eight and nine days after infection, appeared larger than those from nonresistant animals sacrificed at the same time. This appearance is due to emphysema in the lungs of the resistant guinea pigs. A similar condition was not very apparent in the lungs of the nonresistant animals until later (10 to 12 days after infection) as will be seen from an examination of the photographs of the lungs Nos. 109 to 115 in Fig. 4. A close examination of the photograph of the lung from guinea pig No. 113 will reveal a finger impression on the left posterior lobe that was made purposely to illustrate the emphysematous condition. It was found also that the larvae were larger, on the average, in the lungs of the nonresistant animals than in the lungs of the resistant animals on any one day, except the 12th, following infection (Table I). This will be discussed later following the outline of another similar experiment.

FIG. 4. Gross appearance of lungs from resistant and nonresistant guinea pigs 6 to 12 days after infection. Nos. 69 to 95—lungs from resistant guinea pigs. Nos. 109 to 135—lungs from nonresistant guinea pigs. No picture available of Nos. 75, 76, 78, 79, and 116 to 119. Appearance 6 days after infection. Severe congestion in lungs of Nos. 132 to 135, some congestion in lungs of Nos. 92 to 95. Appearance 7 days after infection. Some congestion in lungs of Nos. 128 to 131, very little congestion in lungs of Nos. 88 to 91. Appearance 8 days after infection. Severe congestion in lungs of Nos. 124 to 127, no congestion but some emphysema in lungs of Nos. 84 to 87. Appearance 9 days after infection. Congestion in lungs of Nos. 120 to 123 less severe than previously and some emphysema. Emphysema marked in lungs of Nos. 80 to 83. Appearance 11 days after infection. Marked emphysema in lungs of Nos. 109 to 112, emphysema slight in lungs of Nos. 70 and 73, lungs of 74 and 77 have almost normal appearance. Appearance 12 days after infection. Marked emphysema in lungs of Nos. 113 to 115, slight emphysema in lungs of Nos. 69 to 72.

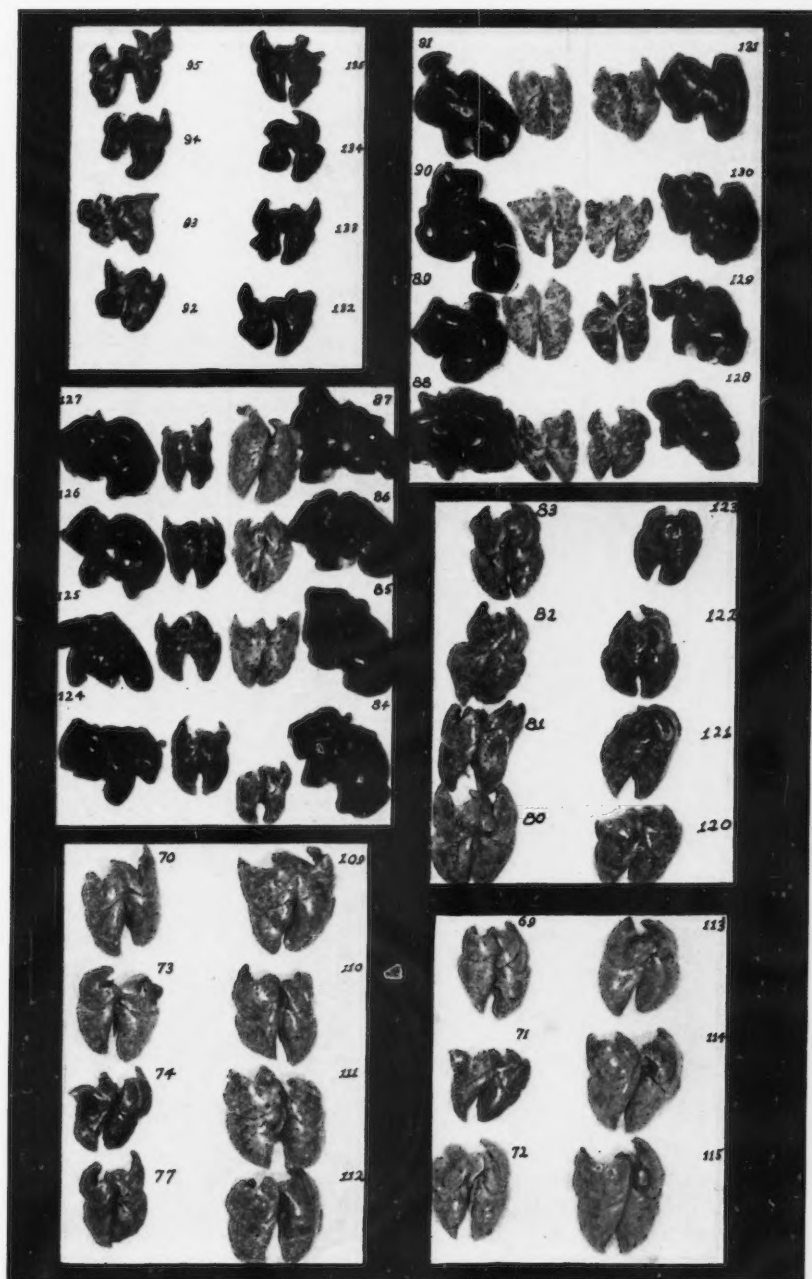


FIG. 4

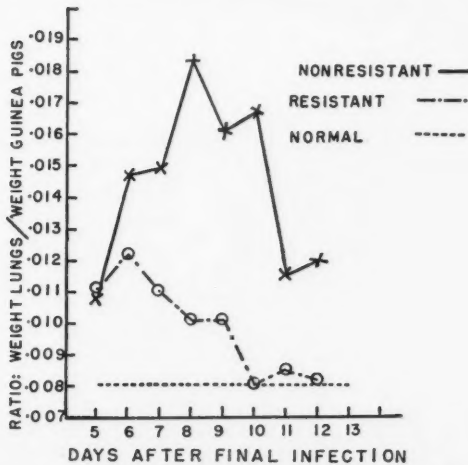


FIG. 8. The relationship between the weights of the lungs in resistant and nonresistant guinea pigs and the weights of the animals during the course of infection with *Ascaris*. The weights on different days following infection are the averages for the three or four animals that were sacrificed on successive days following infection.

It is obvious from these observations that guinea pigs that have been infected previously with *Ascaris lumbricoides* are somewhat resistant to subsequent infections. It will be observed also (Fig. 3) that the average percentage of eosinophiles in the guinea pigs in the resistant group increased more rapidly than in those that had been infected for the first time.

FIG. 5. The lungs of guinea pigs that had repeated infections, a single infection, and injections of antigen.

Appearance 6 days after infection. Nos. 156 to 158 (IA) and 171, 172, 212 (IB), and 185 to 187 (II) show marked congestion although less in IA. Lungs of guinea pigs Nos. 198 to 199 (IIIA) and Nos. 209 to 211 (IIIB) appear almost normal.

Appearance 7 days after infection. Nos. 153 to 155 (IA), 168 to 170 (IB), and 182 to 184 (II) show marked congestion although slightly less in IA. Nos. 196 to 197 (IIIA) and 206 to 208 (IIIB) appear almost normal.

Appearance 8 days after infection. Nos. 150 to 152 (IA), Nos. 165 to 167 (IB), and 179 to 181 (II) show some congestion and emphysema especially in II. Nos. 193 to 195 (IIIA) and 203 to 205 (IIIB) show some emphysema.

Appearance 9 days after infection. Nos. 147 to 149 (IA), 162 to 164 (IB), and 176 and 178 (II) show slight congestion and some emphysema. Nos. 188, 191, 192 (IIIA), and 200 to 202 (IIIB) appear normal.

Appearance 10 days after infection. Nos. 144 to 146 (IA), 159 to 161 (IB), and 173 to 175 (II) show emphysema and slight congestion.

FIG. 6. Gross appearance of lungs, seven days after infection, from guinea pigs that were (a) resistant as a result of previous infection (Nos. 428 to 430, 434 to 436); (b) infected for the first time (Nos. 437, 438, 440); (c) receiving injections of liver extract prepared from normal guinea pigs (Nos. 441 to 443); (d) receiving injection of liver extract prepared from resistant guinea pigs (Nos. 444 to 446).

FIG. 7. Appearance of lungs, seven days after infection, from guinea pigs that had (a) received injections of 'normal' serum at the time of infection (Nos. 542 to 550); (b) received injections of 'resistant' serum at the time of infection (Nos. 551 to 559); (c) received no injections (Nos. 560 to 568).

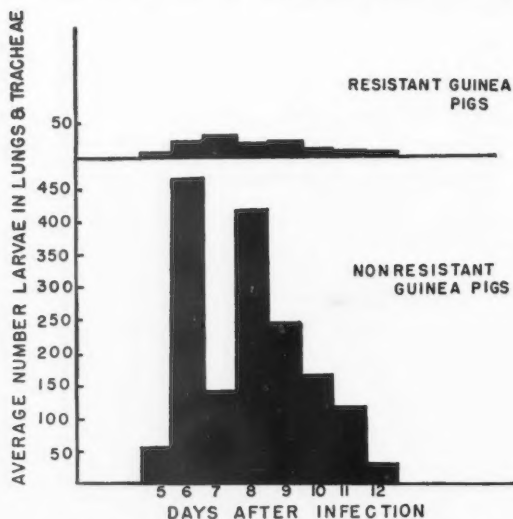


FIG. 9. Average number of *Ascaris* larvae recovered from the lungs and tracheae of four resistant and four non-resistant guinea pigs on successive days following infection, except on the 12th day on which only three animals in each group were examined.

Resistance in Guinea Pigs Following Repeated Infections and in Those Showing a High Eosinophilia Resulting from Injection of *Ascaris* Antigen

Three groups of guinea pigs were selected so that there were 30, 30, and 24 animals respectively, in each group. The animals in Group I received injections of *Ascaris* antigen as shown in Fig. 10, those in Group II were kept as controls; each of those in Group III was fed 8000, 15,000 and 15,000 *Ascaris*

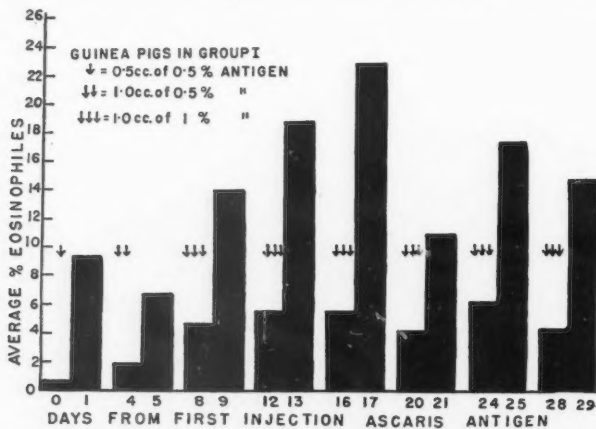


FIG. 10. The average eosinophilia in 10 guinea pigs before and after repeated intraperitoneal injections of phenolized *Ascaris* antigen.

eggs at intervals of 11 days respectively, as indicated in Fig. 11. Forty days after the experiment was begun each of the guinea pigs was fed approximately 25,000 *Ascaris* eggs. Fifteen of the animals in Group I (IA) and 12 of the animals in Group III (IIIA) received six daily injections of 0.5 cc. antigen beginning at the time of this infection.

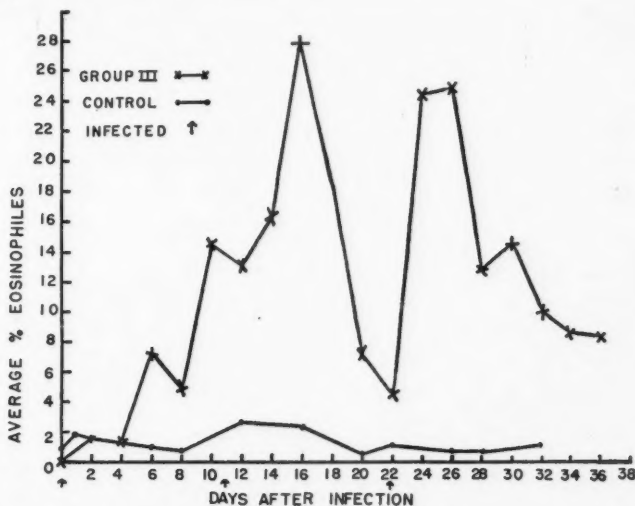


FIG. 11. Average percentage of eosinophiles in 24 guinea pigs following repeated infections with *Ascaris* at intervals of 11 days, together with the average for five guinea pigs that were not infected.

A high eosinophilia was produced by infections (Figs. 11 and 12). The injections of antigen also produced an eosinophilia (Fig. 10) but four days later it was considerably lower. However, a gradual rise in the eosinophile level was noticed following repeated injections for 16 days (Fig. 10). It will be observed that the average percentage of eosinophiles in those guinea pigs (Group IA) that received injections of antigen at the time of infection, as well as a previous series of injections, remained at a higher level than in the guinea pigs (Group IB) that did not receive injections at the time of infection (Fig. 12). The guinea pigs (Group IIIA) that had been repeatedly infected and that had received also injections at the time of their final infection, showed an initial rise in eosinophiles above that in the animals (Group IIIB) that did not receive injections, but subsequently the eosinophilia was higher, on the average, in the animals in the group IIIB.

The susceptibility of the guinea pigs to infection was compared by sacrificing three or four of the animals in each group on the 6th to 10th days inclusive following this final infection. The gross appearance of the lungs of the guinea pigs was recorded in kodachrome pictures (Fig. 5).

The largest number of larvae was recovered from the guinea pigs in Group II, i.e., the control group, which had been infected only once (Table II). Few

TABLE II

AVERAGE NUMBER OF LARVAE RECOVERED FROM LUNGS OF RESISTANT AND NONRESISTANT GUINEA PIGS AND THOSE RECEIVING INJECTIONS OF ANTIGEN

Days, after infection	Group IA, antigen injections prior to and during infection	Group IB, antigen injections prior to infection	Group II, control	Group IIIA, infected previously and received antigen	Group IIIB, infected previously
6	13	23	21	1	1
7	32	35	69	1	1
8	43	138	393	—	1
9	33	105	93	1	1
10	137	61	218		

parasites were found in the animals in Group III, i.e., those that had received three infections, the first 40 days, and the last 18 days, before the final one. There was no significant difference in the number of parasites found in the animals in Group IIIA, which had received injections of antigen, compared with those in IIIB, which did not receive antigen. Fewer parasites were recovered, on the average, from the guinea pigs in Group IA than from those in Group IB. The former had received injections of antigen for six days following their infection as well as a series of eight injections at four day intervals at the beginning of the experiment. The animals in Group IB, on the other hand, had received only the series of injections at the beginning. A comparison of the gross appearance of the lungs from the guinea pigs in the different groups (Fig. 5) with the number of larvae recovered (Table II) illustrates the relationship between gross lesions and the number of larvae present.

The results confirm those of the previous experiment and show that guinea pigs that have been infected previously with *Ascaris lumbricoides* show a strong resistance to subsequent infection acquired 18 days later. There was also a slight indication that injections of *Ascaris* antigen, especially when administered before and during infection, may have produced some resistance in the guinea pigs receiving them. The animals that had received injections at the time of infection (IA) were more resistant than those that had not received these injections (IB). The resistance in the animals that had received injections of antigen, i.e. Group IA and IB, was not as complete as in those animals that had been immunized by reinfection, i.e., Group III. The high eosinophilia (Figs. 11, 12) in the resistant guinea pigs (Table II, Fig. 5) is apparent. It is evident also, from a comparison of the eosinophilia, in the animals in Groups IB and II and the number of larvae recovered from the two groups (Table II) that other factors are involved in resistance. The animals in Group IB showed some resistance and yet the eosinophile level was close to that observed in the animals (Group II) that were presumably nonresistant to *Ascaris* infection.

Duration of Resistance and Effects of Injections of *Ascaris* antigen

Eighty-four guinea pigs ranging in weight from 250 to 500 gm. were divided into four groups containing 24, 24, 24, and 12 pigs respectively. The guinea pigs in the different groups were infected with *Ascaris* eggs as indicated in

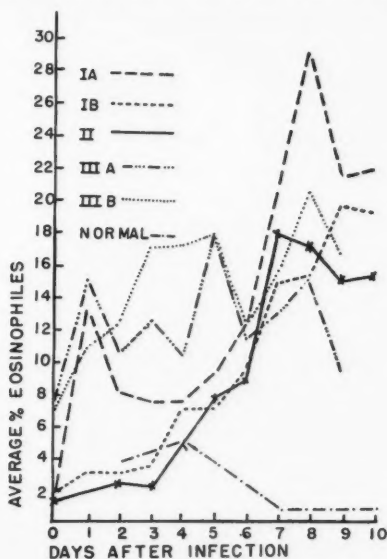


FIG. 12. A comparison of the eosinophilia produced by infection with *Ascaris* in guinea pigs that had received the following treatment prior to this infection.

Group IA — injections of antigen prior to, and during infection;

IB — injections of antigen prior to infection;

II — control, no previous infection;

IIIA — previous infections, as well as injections of antigen at the time of final infection;

IIIB — previous infections.

The averages are based on counts from five animals in each group except on the last day when there were only three animals left in each group.

Fig. 13. The animals in Group A were given three immunizing infections at intervals of nine and seven days respectively and four of the animals in this group received a fourth infection one week later. The animals in Group B were given a single infection and nine days later four of them were given a second infection comparable to that given to the four animals in Group A. The guinea pigs in Group C constituted the control group. Four of these animals were given a single infection at the same time as the four animals in Groups A and B received their final infections. Four of the animals in Group D were given a single infection as well as 1 cc. intraperitoneal injections of 1% antigen on the day prior to infection and for the following seven days. The four pigs in each group were sacrificed eight days after these infections.

Four additional guinea pigs in Groups A, B, and C were infected at intervals of 3, 5, 7, 10, and 15 weeks, respectively, following the final immunizing infections that had been given to the animals in Groups A and B. Guinea

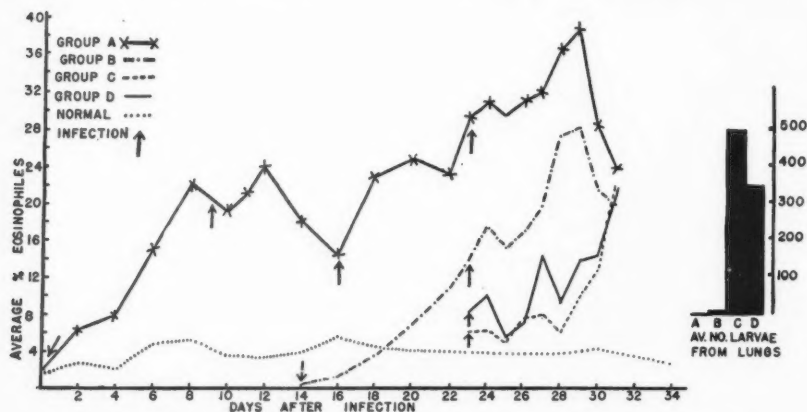


FIG. 13. Eosinophilia in guinea pigs in which infections were superimposed and a comparison with that observed in guinea pigs infected for the first time and in those receiving injections of antigen. The average number of larvae recovered from the lungs of four of the animals in each group, eight days after their final infection, is shown also.

pigs from Group D were infected at the 7 and 15 week intervals. The animals in this group received intraperitoneal injections of antigen at four day intervals and daily during infection. The four guinea pigs in each group were sacrificed eight days after infection.

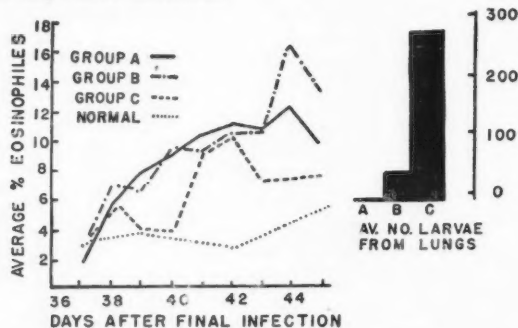


FIG. 14. Comparison of the eosinophilia produced by infection in nonresistant guinea pigs and in guinea pigs that were resistant as a result of infections given three weeks previously. The average number of larvae recovered from the lungs of four of the animals in each group, eight days after their final infection, is shown.

An eosinophilia was associated with the infections (Figs. 13 to 18). It was more pronounced when infections were superimposed (Groups A and B, Fig. 13). This resembles the result obtained by Bachman and Rodriguez

Molina (1) with superinfections with *Trichinella spiralis*. It differs from the results of the work by Brown and Otto (3), with hookworms, in which they found that the addition of new worms did not necessarily induce an eosinophilia. In some instances (Fig. 16), the eosinophile level was high in the vaccinated guinea pigs, but in other cases it was similar to that in the unvaccinated animals (Fig. 18).

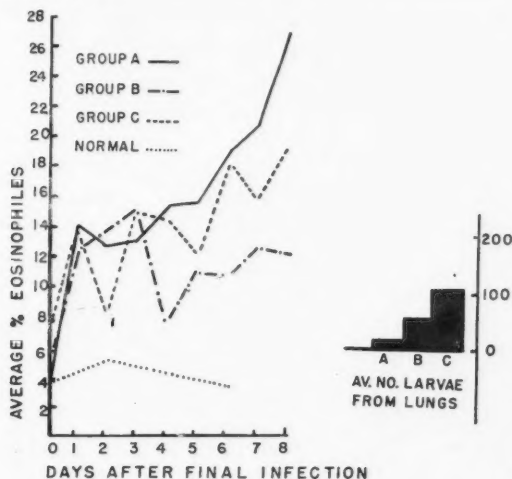


FIG. 15. Similar to Fig. 13—except guinea pigs in resistant group had received last immunizing infection five weeks previously.

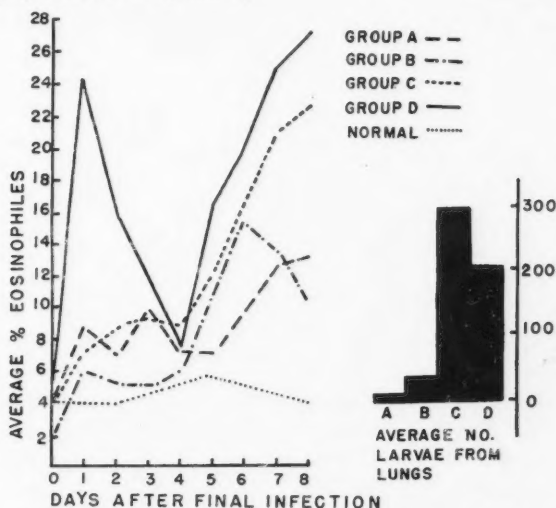


FIG. 16. Similar to Fig. 13—except guinea pigs in resistant group had received last immunizing infection seven weeks previously.

The average number of larvae recovered eight days after infection from the lungs of the guinea pigs that had received respectively, three immunizing infections (Group A), one immunizing infection (Group B), no previous infection (Group C), and injections of antigen (Group D), are given in Table III and illustrated in Figs. 13 to 18.

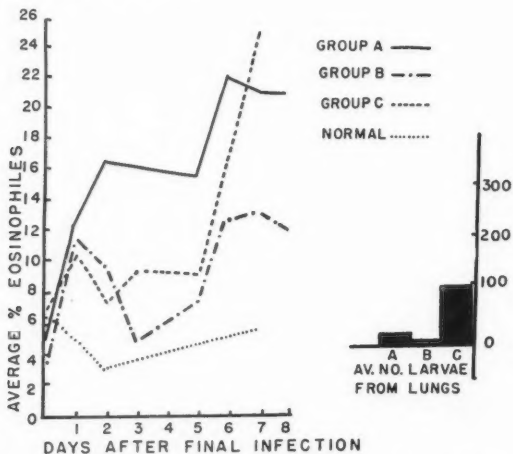


FIG. 17. Similar to Fig. 13—except last immunizing infections had been given 10 weeks previously.

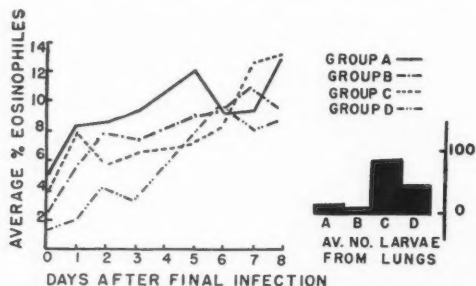


FIG. 18. Similar to Fig. 13—except last immunizing infection had been given 15 weeks previously.

It would appear from the number of larvae recovered from the lungs (Table III), that guinea pigs that received three infections at intervals of seven to nine days (Group A) were more resistant to subsequent infections than those that had received, previously, a single infection (Group B). It is also apparent that guinea pigs were partially resistant to infection with *A. lumbricoides* for at least 15 weeks following a previous infection. (The smaller number of larvae recovered from the pigs in the latter part of the experiment is probably due to fewer living larvae in the eggs fed. Approximately the same number of eggs from the same lot were fed throughout the experiment. It is possible,

TABLE III

AVERAGE NUMBER OF LARVAE RECOVERED, EIGHT DAYS AFTER INFECTION, FROM LUNGS OF FOUR GUINEA PIGS IN GROUPS A, B, C, AND D, AT INTERVALS FOLLOWING IMMUNIZING INFECTIONS GIVEN TO GROUPS A AND B AND ANTIGEN INJECTIONS TO GROUP D

Weeks after last immunizing infection	Average number of larvae recovered			
	Group A, three immunizing infections	Group B, single immunizing infection	Group C, control	Group D, received antigen
1	+	5	507	349
3	2.5	23	280	
5	16	51	105	
7	9	35	298	208
10	25	8	96	
15	16	6	92	48

too, that the age and size of the animals may have had some influence on the result, although an effort was made to minimize such an effect by selecting the larger animals at the beginning of the experiment.) Injections of *Ascaris* antigen gave the pigs relatively insignificant protection against the parasites.

It is apparent from an examination of the results given in Figs. 13 to 18 that *Ascaris* infection produces a marked eosinophilia in guinea pigs. Moreover it reaches higher levels in guinea pigs that have been infected repeatedly at short intervals. It is evident, also, that the eosinophilia is not related directly to the resistance, although such an erroneous conclusion might have been made if the experiment had included only the results shown in Fig. 13. Injections of antigen may produce an eosinophilia but they do not produce a resistance comparable to that which is present following infection. Moreover the eosinophilia may be similar in the animals in different groups but the animals in one group may be much more resistant than those in another (Figs. 14 and 18).

Localization of Resistance

Two groups of guinea pigs, with 16 animals in each, were selected. The guinea pigs in one group were infected twice; those in the second group received a single infection. Two pigs from each group were sacrificed daily for eight days beginning 16 hr. after infection.

The number of larvae recovered from the animals in the two groups (Table IV and Fig. 19) provide additional evidence of the resistance that is produced by infection. It appears from the number of larvae recovered from the livers and lungs, that the body defences, in resistant animals, act against the larvae even before they reach the liver as fewer larvae were recovered from the livers of resistant animals than from those of nonresistant animals. It is evident also that more larvae reached the livers in resistant animals than succeeded in reaching the lungs. This suggests that the body defences are strongly mobilized to act against the larvae before they reach the lungs.

TABLE IV

AVERAGE NUMBER OF LARVAE IN LIVERS AND LUNGS OF TWO RESISTANT AND TWO NONRESISTANT GUINEA PIGS ON SUCCESSIVE DAYS FOLLOWING INFECTION

Time after infection	Resistant		Nonresistant	
	Liver	Lung	Liver	Lung
Hours 16	36	0	13	0
Days 2	30	0	86	0
3	15	1	112	0
4	26	1	84	18
5	24	0	1	46
6	2	2	2	142
7	2	2	1	38
8	0	2	1	112

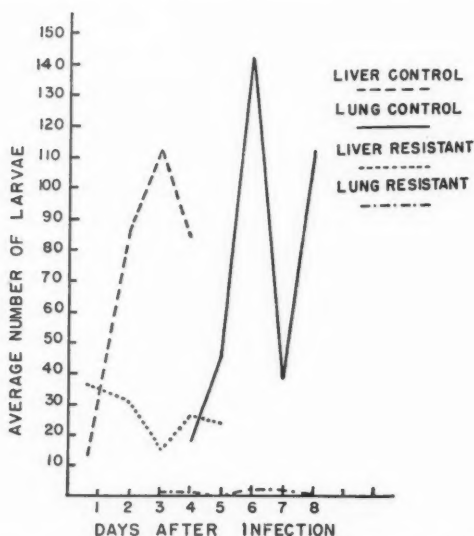


FIG. 19. Average number of larvae recovered from livers and lungs of two resistant and two nonresistant guinea pigs on successive days following infections.

Lungs of all the guinea pigs that were killed during the first three days following infection appeared normal. The livers in the resistant guinea pigs contained many small lesions similar to those described by Schwartz and Alicata (13) and Oldham and White (10) in hogs; similar lesions were scarcely noticeable in the control guinea pigs during the first three days following infection and were never as numerous as in the livers of guinea pigs from the resistant group.

The measurements of the larvae obtained from the livers and lungs of resistant and nonresistant animals were combined with similar data from a previous experiment (Table I) to construct Fig. 20. Each point shown on

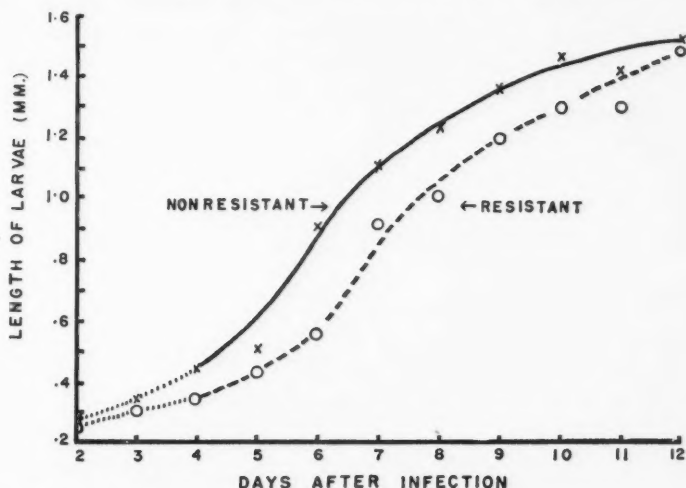


FIG. 20. Average size of larvae in livers and lungs of resistant and nonresistant guinea pigs on successive days following infection. Measurements made two, three, and four days after infection on larvae recovered from livers, the rest on larvae from the lungs.

Fig. 20 is based on the average of at least 14, and usually 20 or more, measurements except that for 12 days in the resistant group, which is the average of six measurements. It is apparent from these results that, except on the 12th day following infection, the larvae recovered on any one day were, on the average, smaller in resistant guinea pigs than in those that were not resistant to infection.

Effect of Liver Extract from Resistant Guinea Pigs

Five groups, with three guinea pigs in each, were treated as follows:

Guinea pigs in Groups I and II were given two infections 14 days apart.

Guinea pigs in Group III were infected once, and thus constituted the control group.

Guinea pigs in Group IV were infected once and received 1 cc. intraperitoneal injections of 'normal' liver extract beginning the day prior to infection and continuing for eight days.

Guinea pigs in Group V were infected once and received injections of 'resistant' liver extract at the same time and in the same quantities as that given to the pigs in Group IV.

The pigs in each of the groups were sacrificed by stunning and cutting the jugular veins seven days after infection. The gross appearance of the lungs

from each of the animals is shown in Fig. 6. It will be observed that the lesions were pronounced in the lungs of the pigs that were infected only once and in those that were infected once and received injections of normal liver extract. The lesions were least extensive in the animals that had been infected twice. The lungs of the pigs that received resistant liver extract were more congested than those from pigs that had been infected twice but less congested than those from animals that received normal liver extract. Most larvae were recovered from the lungs that showed the most congestion (Table V).

TABLE V

AVERAGE NUMBER OF LARVAE RECOVERED FROM LUNGS OF GUINEA PIGS SEVEN DAYS AFTER INFECTION

Group	No. pigs in group	Description	Average no. larvae
I	3	Resistant as result of infection two weeks previously	1
II	3	Resistant as result of infection two weeks previously	1
III	3	Controls—single infection	434
IV	3	Received liver extract from normal pigs	146
V	3	Received liver extract from resistant pigs	76

It appears from this experiment that resistant liver extract given intraperitoneally to guinea pigs at the time of infection gave them some protection.

Effect of Serum from Resistant Guinea Pigs

Nine guinea pigs were given 2 to 3 cc. of 'resistant' serum intraperitoneally for five days. Nine pigs were given similar amounts of 'normal' serum in the same way. Each pig was fed several thousand *Ascaris* eggs two days after receiving the first injection of serum. A third group of nine pigs was kept as control and infected at the same time as the pigs receiving sera. The pigs were sacrificed seven days after being infected. The lungs (Fig. 7) of the guinea pigs that received resistant serum were less congested than the others. The number of larvae recovered from the guinea pigs in the different groups is given in Table VI.

The difference between the means of the number of larvae recovered from animals that received resistant serum and from those in the other groups was significant as shown by application of the *t* test. The variability in the number of larvae recovered from the guinea pigs receiving normal serum is a striking, although unexplained feature.

It would appear from these experiments that some passive resistance may be conferred on guinea pigs, at the time of their infections, by intraperitoneal

TABLE VI

NUMBER OF LARVAE RECOVERED FROM GUINEA PIGS THAT RECEIVED 'RESISTANT' SERUM, 'NORMAL' SERUM, AND NO SERUM AT THE TIME OF INFECTION

Resistant serum	Normal serum	Control
17	157	148
4	372	84
3	123	89
5	222	96
1	54	94
49	44	75
5	206	137
14	9	162
12	16	91
Av. 12	Av. 134	Av. 108

injections of sera from guinea pigs that were infected with the parasite three weeks previously.

The average percentage of eosinophiles in the peripheral blood of the guinea pigs receiving resistant serum was higher than in those animals that received injections of normal serum, but the former showed a marked drop on the eighth day. It should not be concluded however, that this difference in the eosinophile level is, itself, responsible for the resistance observed.

Discussion

The results of the experiments outlined above confirm those of other investigators who have found that animals that are heavily infected with *Ascaris* have marked symptoms and may even succumb as a result of the infection. The congestion and damage to the lungs were striking pathological features but unlike the congestion resulting from certain types of respiratory infections no elevation of temperature was observed. Blackie (2), from his histopathological studies on *Parascaris equorum*, concluded that two factors, (1) mechanical and (2) toxic, were responsible for the lesions produced. In the lung the mechanical damage overshadowed the toxic effects that were associated, he thought, with the hepatic and renal lesions that were responsible for death. Roberts (12) arrived at rather similar conclusions from his studies on *Ascaris lumbricoides*.

A high eosinophilia was associated with heavy infections and this eosinophilia reached even higher levels when second, third, and fourth infections were superimposed upon the first before the number of eosinophiles had returned to normal. A marked increase in basophiles, which replace the eosinophiles in foxes, was observed by Kennedy and Law (7) following the administration of *Ascaris* eggs to these animals. The increase appeared to be directly proportional to the number of eggs given. This is of interest because in the present experiments no direct relationship was found between the number of eggs fed and the number of larvae recovered from the tissues.

Injections of *Ascaris* antigen also caused an increase in the number of eosinophiles but the number returned to the normal level more rapidly than was the case following infection. In a number of infections the eosinophilia developed more rapidly in guinea pigs that had been infected previously. This fact suggests that the eosinophiles may have an important role in the resistance that was demonstrated quantitatively to exist in guinea pigs that had received multiple infections. The importance of the eosinophile in this connection is suggested further by the fact that, in a number of animals, the resistance was most marked in those that showed the highest eosinophilia.

The present experiments suggest, however, that the eosinophiles are not solely responsible for the resistance observed, for injections of antigen produced a high eosinophilia, but rendered guinea pigs only slightly resistant to infection. Moreover, evidence was produced to show that a degree of passive resistance could be conferred on guinea pigs by injecting large quantities of serum from resistant animals or extracts from livers of resistant animals. The lesions observed in the livers together with the number of larvae recovered from the livers and lungs of resistant animals suggests further that substances, or cells, or both, in the liver play an important part in the resistance observed. Some of the cells involved in the liver are probably eosinophiles but other factors are likely concerned also. Other work on the role of eosinophiles and other factors in resistance in ascariasis is in press (Sprent and Chen (14)). It may be that the eosinophiles are a result, rather than a cause, of resistance.

The high eosinophilia associated with infection in resistant animals together with the gross appearance of their lungs suggest a possible relation between resistance to *Ascaris* in guinea pigs and sensitivity. The emphysema that appeared in the lungs of guinea pigs following infection with *Ascaris* and that was manifest sooner in resistant animals than in those nonresistant to infection also supports this view. It was found that by the time the emphysema appeared in the nonresistant animals, namely 8 to 12 days after infection, that the animals had developed a resistance to the parasite as shown by feeding a second dose of *Ascaris* eggs at this time. This resistance could be due to antibodies as their production would be expected about one to two weeks after infection. The decline in resistance that was noticed in animals infected 3 to 15 weeks after their last immunizing infection could be related to a decline in antibodies. The passive resistance observed as a result of injections of serum from guinea pigs infected three weeks previously could be related also to antibodies.

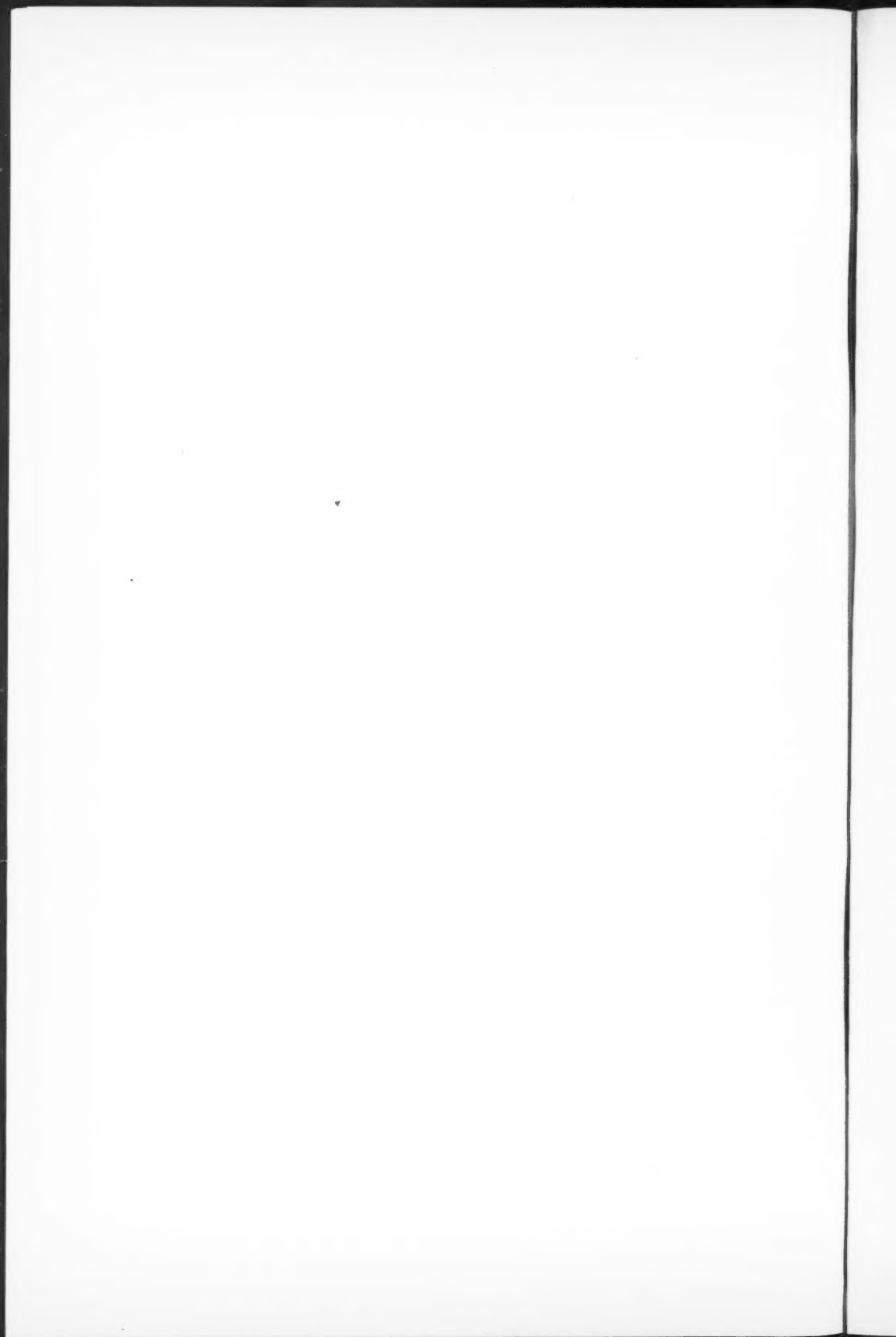
The resistance appeared to affect the size of the larvae to be found in the tissues on any one day. The difference in size was apparently a result of a difference in rate of growth rather than a difference in the rate of migration of the larvae through the tissues as it will be observed from Fig. 9 that the maximum number of larvae was found in the lungs of resistant animals about the same time as in nonresistant animals.

Acknowledgments

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References

1. BACHMAN, G. W. and RODRIGUEZ MOLINA, R. Resistance to infestation with *Trichinella spiralis* in hogs. The eosinophilic and precipitin response. *Am. J. Hyg.* 18 : 266-294. 1933.
2. BLACKIE, W. K. Histological observations on experimental ascariasis. *J. Helminthol.* 8 : 93-102. 1930.
3. BROWN, H. W. and OTTO, G. F. The differential leukocyte count associated with hook-work infection. *Am. J. Diseases Children*, 61 : 727-733. 1941.
4. DEBOER, E. Experimentelle untersuchungen über *Ascaris lumbricoides* des Menschen und des Schweinen. *Z. Infekt. Hyg. Haustiere*, 48 : 248-279. 1935.
5. FALLIS, A. M. Use of the Waring Blendor to separate small parasites from tissues. *Can. J. Pub. Health*, 34 : 44. 1943.
6. HADWEN, S. Ascariasis in horses. *J. Parasitol.* 12 : 1-10. 1925.
7. KENNEDY, A. H. and LAW, R. G. The differential blood changes in ascariasis in foxes. *Can. J. Research*, 12 : 277-285. 1935.
8. KERR, K. B. The cellular response in acquired resistance in guinea pigs to an infection with pig *Ascaris*. *Am. J. Hyg.* 27 : 28-51. 1938.
9. MORGAN, D. O. Some observations on experimental ascariasis in pigs. *J. Helminthol.* 9 : 121-128. 1931.
10. OLDHAM, J. N. and WHITE, E. G. Chronic focal interstitial hepatitis in the pig: its experimental production by feeding *Ascaris* eggs. *J. Comp. Path. Therap.* 54 : 1-15. 1944.
11. RANSOM, B. H. and CRAM, E. B. The course of migration of *Ascaris* larvae. *Am. J. Trop. Med.* 1 : 129-155. 1921.
12. ROBERTS, F. H. S. The large roundworm of pigs *Ascaris lumbricoides* L. 1758. *Bull. 1*, Animal Health Station, Yeerongpilly, Queensland, Australia. 1934.
13. SCHWARTZ, B. and ALICATA, J. E. *Ascaris* larvae as a cause of liver and lung lesions in swine. *J. Parasitol.* 19 : 17-24. 1932.
14. SPRENT, J. F. A. and CHEN, H. H. Immunological studies on mice infected with larvae of *Ascaris lumbricoides*. *J. Infectious Diseases*. In press.
15. WAGNER, O. Immunisierungsversuche bei experimenteller *Askaris* infektion der Maus. *Z. Immunitäts. exptl. Therap.* 78 : 372-382. 1933.



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